JOHANNES MÜLLER.

AN INTRODUCTION TO THE STUDY OF SECRETION

BY

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"Another error is an impatience of doubting and a blind hurry of asserting without a mature suspension of judgment . . . if we begin with certainties, we shall end in doubts ; but if we begin with doubts, and are patient in them, we shall end in certainties."

BACON : *Advancement of Learning.*

"Es ist schwer, genau und fein zu beobachten, aber noch schwerer, aus dem Beobachteten nicht mehr zu folgern, als es enthält."

EHRENBERG.

PREFACE

The following pages consist of a report in slightly expanded form of a series of eight University Lectures given at the Middlesex Hospital Medical School during the Winter Session 1922-23.

The account of the various subjects treated is not intended to be complete. It is hoped that the book will be useful if only from the consideration that the different topics here brought under review have not previously been discussed within the space of a single volume.

The bibliography is of course selected, and not exhaustive. From it, however, most of the rest of the literature could be collected. Of the various works consulted special mention ought to be made of the article on Secretion in Bayliss' *General Physiology*. The article suggested many points for presentation and provided a basis for the literature.

One of the main objects has been to examine the main facts of secretion and " internal secretion " to see how far they may legitimately be included in a common category. If the results are somewhat inconclusive I must urge that this is not altogether my fault, but arises from the imperfection of our knowledge.

It is anticipated that the book will be found useful to students and medical men and teachers who wish to obtain a bird's-eye view of what is known of the processes of secretion.

I have to thank Professors A. E. Boycott and A. T. Cameron, Dr. W. Cramer, and Mr. J. H. Woodger for criticisms and suggestions, and Mr. Samson Wright for reading my manuscript and assistance in the preparation of the book for the Press. I have also to thank the Director of the Imperial Cancer Research Laboratory for permission to use some blocks of illustrations published by the Fund.

SWALE VINCENT.

Middlesex Hospital Medical School,
January, 1924.

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CHAPTER I

INTRODUCTION

In the following pages some account of the anatomy and histology of glands and secreting cells will be given. This will be followed by a discussion of the physiological processes of secretion – that is to say, of the chemistry and physical chemistry of secreting tissues. Finally the more general aspects of what is usually termed "internal secretion" will be presented. The object of such presentation will be to investigate in how far we may regard the processes of "internal secretion" as comparable with those of ordinary or "external" secretion.

History

The term "secretion" has long been associated with structures known as glands. In the earliest days the term gland was employed in a very loose manner, and the same applies to the word "secretion." For a long time anatomists regarded only the external form and general appearance of various organs and all rounded, white or reddish organs were called "glands." The first detailed description of the structure of glands was given by Malpighi [91] in 1665, who held that the elementary parts of all glands – the "acini" – have the same structure as the simple follicles and the "conglomerated follicular glands"; that is, that they consist of minute roundish sacs which receive the secretion from the blood-vessels, and pour it into the efferent ducts. But Malpighi did not realize that each of his "elementary parts" is an accumulation of many of the real elementary parts. In 1661 he had used a microscope and had discovered the capillaries, but even in 1665 had apparently not discovered the individual gland cells.

Ruysch [108] in 1696 used the method of injection and succeeded in showing that the so-called " follicles " contain a vast number of tiny blood-vessels, but he imagined that the proper substance of glands consists solely of blood-vessels and that the smallest vessels terminate by opening directly into the ducts. Haller [66] lent his support to this doctrine. It was Johannes Müller [99] who proved conclusively that the secreting canals in all glands form an independent system of tubes and that the blood-vessels only form a capillary network on their walls and in their interstices. He used the method of injection, as did Ruysch, but his investigations showed that whenever during injection of the ducts whether of the liver or kidney the blood-vessels become filled, the minute ducts themselves did not receive any injection ; consequently, that extravasation must have taken place.

It would not be profitable to pass in review the various theories of secretion discussed by Müller, but an admirable summary of the state of knowledge in Müller's time will be found in his *Elements of Physiology* [99] as also in the articles on Secretion by Carpenter [32] and on Glands by Grainger [64] in Todd's *Cyclopædia.*

During more recent years a vast amount of work has been carried out upon various kinds of secretory processes. The various processes which are called secretory differ in nature so widely that a resumé of the history cannot be attempted in the different branches. In what we may call the most typical cases of secretion (such as those of the salivary glands and the pancreas) the most striking observations have been made by Ludwig, Heidenhain, Claude Bernard and Langley. Ludwig [88] in 1851 discovered in the dog secretory fibres for the submaxillary gland in the lingual branch of the fifth nerve, and was the first to show by experiment on the same gland in the dog that the secretory pressure may overpass considerably the blood-pressure.[89] Bernard [18] showed that the secretory fibres for the submaxillary glands come from the chorda tympani and hence from the facial nerve. The changes in the histological appearance of the secreting cells during secretion were first described by Heidenhain.[70] But Langley,[81] using fresh material, gave a clearer account of what really occurs. The work of more recent writers will be referred to later on.

By the year 1852 a new sort of secretion began to be discussed. The anatomists having decided that certain structures without ducts were, notwithstanding this, essentially glands, began to postulate the flow of secreted material into the blood-stream. The doctrine of internal secretion as it is generally taught to-day was founded by Claude Bernard and Brown-Séquard. How far we are justified in looking upon "internal secretion" as analogous with true or external secretion will be discussed in a later chapter.

CHAPTER II

ANATOMY AND HISTOLOGY OF GLANDS

A true secreting gland is made up of cells of a special epithelial character, and forms a product—the secretion—of a fluid or semi-fluid nature, which is poured out upon an epithelial surface such as the skin or a mucous membrane.

The primitive type of a secreting structure is a layer of epithelial cells (sometimes placed on a basement-membrane) with ramifying blood-vessels and lymph-spaces. The mucous membrane of the alimentary canal from the stomach to the rectum may be considered to represent such a simple type, if we neglect the villi and the crypts, and the serous and synovial membranes may be regarded as examples of simple forms of secreting apparatus.

But in the formation of "glands" the surface may become variously folded and involved. There are two modes by which the secreting surface may be increased in extent, namely by *protrusion*, or by *recession*. Examples of the first method are found in the Haversian fringes of the synovial membrane and the choroid plexus. The most usual method is by recession. The layer of cells becomes invaginated, and we get as a result a *tubular* or a *saccular gland*. If the structure thus formed is simple (i.e. unbranched) the blind end is often somewhat enlarged and is called the "fundus" of the gland. Such glands may be coiled as in the case of the sweat-glands. Simple straight tubular glands are found in the stomach of various animals.

The secretory portions of the gland may divide, forming branched tubular glands. The branching may occur again and again until a very complicated structure is produced. This state of affairs constitutes what is known as a *compound tubular* or *compound saccular* (*racemose*) gland. In such glands the terminal portion of the tubes or "alveoli" are

the secretory portions, while the tubes leading to the exterior are the "ducts."

By the use of the methods of reconstruction and teasing it has been discovered that there is a very great variation in the shape and arrangement of the alveoli, even in glands which are otherwise allied in structure. The "serous" glands are the most typical compound racemose glands. The "mucous" gland are often described as *tubulo-racemose*. An example of a compound tubular gland is found in the kidney. In some cases the secretory tubules may anastomose with each other forming a *reticulated tubular gland* (liver).

Blood-vessels of glands. The blood-vessels of a gland form a capillary network in each lobule between and around the alveoli, but they do not come into contact with the basement-membrane, and nowhere therefore, except in the liver, penetrate between the secreting cells. Capillary vessels are also distributed to the connective-tissue framework (Schäfer [111], Flint [52]).

Lymphatics and connective tissue. In all glands, with the exception of the liver, the alveoli are supplied with nutrient plasma from the lymph which bathes the exterior of each alveolus and penetrates the basement-membrane to reach the cells which line it (Schäfer [111]). The blood-vessels and lymphatics are supported by connective tissue which divides up the gland into lobules and passes into the lobules between the alveoli. It forms a network round the alveoli. The efferent lymphatics run with the blood-vessels.

The **Basement-membrane** (*membrana propria*) of a gland is a thin membrane not always continuous, showing nuclei at intervals. It is made up of flattened cells united by their edges or by their processes. According to Flint [53] the meshes of the basket-work are occupied by a delicate membrane which is a condensation of the reticular connective tissue.

Nerve-supply to glands. Both the secreting cells and the blood-vessels of glands are supplied with nerve fibres. The nerves to the blood-vessels are of two kinds, *vaso-constrictors* and *vaso-dilators*, and the nerves to the secreting cells seem also of two kinds (at any rate in some glands). These are

called respectively *trophic* and *secretory*. The first seem to convey impulses which give rise to an increased formation of the products of secretion in the cells. The second appear to stimulate the pouring out of the secretion from the gland. The action of these several kinds of nerve fibres will be referred to again in connection with the secretion of the different glands.

The terminations of nerves have been studied in the pancreas, the salivary glands, the prostate, the skin glands and the mammary gland, and it seems probable that in glands generally there is an epilamellar network which lies on the membrana propria ; from this fine fibres are given off which pass through the membrane and as pericellular threads come in contact with the gland cells. These fibres form no network under the membrana ; they may divide, and end in varicose filaments, the configuration of which differs in different cells. Arnstein [5], Retzius [106] and Huber [76] have paid special attention to the sublingual and submaxillary glands and the latter states that the axis-cylinder branches of the sympathetic cells found by the side of the ducts of the submaxillary gland are grouped into small bundles which accompany the branches of the ducts to the lobules and here form a plexus about the intralobular duct, from which branches are in turn given off which form a second plexus on the membrana propria about the alveoli ; from this plexus fibrillæ penetrate the membrana propria and end on the gland cells. According to the same author the terminal endings of the chorda tympani form a pericellular network about the sympathetic nerve cells of the sublingual and submaxillary ganglia.

Secretory canaliculi. The biliary canaliculi between the cells of the lobule have been known for a long time, and it has been stated that these are in communication with finer channels within the cells. Secretory canals between the secretory cells of many kinds of gland are now recognized and can be displayed by the Golgi silver chromate impregnation method. Such canaliculi have been seen in the parotid gland and in other salivary glands, in the stomach and in the pancreas. It has been suggested that the canaliculi are only present during the actual passage of the secretion.

Plain muscle in glands. Unstriped muscle is found in

the ducts of many glands. It varies in amount and is, for the most part, arranged circularly in the walls of the large ducts.

The General Character of Glandular Cells

Glandular secretory cells vary very considerably in form in the different glands and even in different parts of the same gland. They may be columnar, cubical, ovoidal, polyhedral or flattened. A very usual shape is that of a pyramid, the blunt apex of which points into the lumen of the alveolus, while the base rests upon the basement membrane. The cells are made up of protoplasm containing a centrosome and a nucleus. The latter often lies near the basal membrane, but moves to the centre of the cell after discharge of the secretory products. But most important and most characteristic is the presence of secretory granules in the cell of every kind of secreting gland. The changes in the amount and the disposition of these granules will be referred to later on. Altmann thought that they multiply by fission and that they represent elementary organisms. These views have not been accepted.

The Centrosome. Many cells, including the majority of gland cells, show an area in their interior and usually near the nucleus from which can be seen lines radiating into the protoplasm. This is the *central particle, attraction-particle,* or *centriole.* This again is surrounded by the *attraction-sphere* or *centrosome.* The terminology of these structures is somewhat confused.

Kinoplasm. The protoplasm within and immediately surrounding the centrosome is known as *archoplasm* (Boveri), *kinoplasm* (Strasburger), or *ergastoplasm.* The ordinary granules of secreting cells stain with acid dyes like eosin and acid fuchsin. The kinoplasm on the other hand stains deeply with basic dyes such as methylene blue, anilin blue, neutral red or pyronin. It is said that Janus green brings out the fibrils of kinoplasm in the fresh cell.

It is believed by some observers that the separation of the zymogen granules starts round the nucleus with the production of this basophile "ergastoplasm." From this, granules are gradually formed, and then for a time continue to undergo slight further changes, as is shown by the observation that the staining reaction of those near the base of the cell differs from that of those at the free margin.

Trophospongium. Canaliculi in Cell-Protoplasm. — In many cells of different kinds a network of fine anastomosing canals may be observed. This was first described in nerve-cells, and later in gland cells, epithelial cells of different kinds, the decidua, and the membrane of Descemet of the eye. Special attention has been called by Schafer to the canals in the liver cells where sometimes may be found blood cells in process of disintegration. These liver canaliculi can be injected from the portal vein.

Holmgren states that the canals of the trophospongium open on the exterior of the cells, affording means for the passage of lymph into the interior of the cell-protoplasm, and thinks that they are partly occupied by processes from the surrounding connective tissue cells. Holmgren first used the term "trophospongium." V. Bergen says that there is not always an opening to the outside of the cell and that the canaliculi are not permanent structures, but come and go according to the state of activity of the cell. As Schafer points out, it is clear that in leucocytes and cartilage cells they cannot be occupied by a series of processes derived from connective tissue cells.

Mitochondria.—Certain kinds of granules in cells (according to some writers these are specially related to the kinoplasm) tend to form fibrillæ and so have been named *mitochondria*. Masses of such substance are often called *paranucleus* or *chondromitome*. These structures are not shown by the chromatin fixing fluids, such as Flemming's fluid. The use of acetic acid in any combination renders their display difficult or impossible. Mitochondria may be seen in fresh preparations by means of Janus green and other dyes, but they are best shown by the chrome osmium methods which also bring out the Golgi apparatus (*vide infra*).

Mitochondria are commonly filamentous or rod-like, but they may be granular, dumbbell-shaped or disposed in the form of a network. They are semifluid in nature, and have a tendency to flow together and form large droplets under certain conditions. They have smooth outlines and rounded ends.

Mitochondria are found almost universally in cells. They are most often irregularly distributed throughout the cyto-

plasm. But in the acinous cells of the pancreas they are most numerous in the basal portion of the cell. It is alleged that there is a double polarity in the intestinal epithelium. That is to say, mitochondria are found in two groups at the basal and luminal ends of the cell. This may probably be related to the two functions carried out by the cell, namely secretion and absorption. Again, in the case of the thyroid the polarity is said to be reversed, that is to say, the mitochondria are found only in the part of cell adjoining the colloid mass. Mitochondria do not manifest an independent motility. They disappear in old age. They appear to consist chemically of a phosphatide (lecithin, kephalin) and albumin. It is supposed that the cellular differentiations are formed from the mitochondria, and that they constitute in part the material basis of heredity. They seem to be the starting points for the fat and lipoid metabolism of the cell. Some authors are of the opinion that they dominate all the activities of the cell. Thus it is sometimes suggested that the zymogen granules arise from them.

The Golgi apparatus (*Golgi's internal apparatus*). The Golgi apparatus was first discovered by Golgi about twenty years ago, and afterwards independently by Cajal, in the nerve ganglia as an intra-cellular network around the nucleus —the "apparato interno reticolare." In nearly all cells the Golgi apparatus lies near the nucleus, at any rate at some period in the life of the cell. Here it consists of little rods sticking to the surface of the kinoplasm or attraction-sphere. The Golgi granules (like the mitochondria) are able to divide independently of the other cell elements. The apparatus consists of filaments or rods, straight or curved or forming a network spread out through the cytoplasm of cells. It occurs in all gland cells, and is quite distinct from the mitochondria. When a cell divides the process affects not only the chromosomes, but also the Golgi elements and the mitochondria (Gatenby [57]).

Cell division or { Karyokinesis and Mitosis (chromatin).
Dictyokinesis (Golgi apparatus).
Chondrokinesis (mitochondria).

The mitochondria and Golgi apparatus cannot be shown

by staining after fixation by such agents as corrosive sublimate or Flemming's fluid. The avoidance of alcohol, chloroform, and acetic acid seems to be essential. Several methods and modifications of methods may be employed. The arsenious acid and silver nitrate process of Golgi and the uranium nitrate method of Cajal both give good results. Da Fano [49] recommends (especially for the Golgi apparatus) fixation in cobalt nitrate and formalin, followed by washing and treatment with silver nitrate. After a second washing the tissue is placed in a reducing fluid (hydroquinone, formalin, and sodium sulphite) and after a further washing cut with a freezing microtome.

FIG. 1.—Mammary Gland of Mouse at the tenth day of Pregnancy.

The apparatus is enlarged and less frequently formed of separate portions. (Da Fano.)

FIG. 2.—Mammary Gland of Rat at the fifteenth day of Pregnancy.

Remarkable enlargement of the apparatus with tendency to envelop the nucleus. (Da Fano.)

Quite recently Da Fano [50], following the work of Cajal on the apparatus in the goblet cells of the intestine, has investigated the changes in the mammary gland during the various secretory phases. The apparatus is a constant structure in the epithelial cells of the mammary gland in all stages of activity (see Figs. 1 and 2). In the virginal condition it consists of small networks situated next to the nucleus on the side of the lumen. During pregnancy it greatly hypertrophies

and shows a tendency to surround the nucleus. Throughout lactation the apparatus remains hypertrophic and becomes fragmented and shifted and stretched in various directions. The distortion appears to be due to increased intra-cellular pressure. In the involution after lactation the epithelial cells undergo changes during which the Golgi apparatus appears to become transformed into peculiar roundish, oval, or elongated shapes, limited by a granular or filamentous argentophil material. Most of these cells are cast out with the distorted apparatus. Some remain as the permanent epithelium of the resting gland and in these cells an apparatus is found similar to that of the virginal condition.

Da Fano suggests that the Golgi apparatus of the surviving cells is rebuilt from the fragmented materials of the old one. So in this way the apparatus takes a part in the functioning of the mammary gland.

It is important that investigations of a similar nature should be made upon the apparatus in the secreting cells of other glands.

The Histological Changes in Glands during Secretion

In the year 1875 appeared a very important paper by Heidenhain [69] in which he refers to previous work on secretory changes in glands by Ebstein and by himself. He studied the changes in rest and in activity of the pancreas, as shown by the investigation of fixed material. The observations of Kühne and Lea [80] upon living material in the main confirmed those of Heidenhain in the case of the pancreas.

Langley [81] worked with the parotid gland of the rabbit, rat, cat and dog, the infra-orbital gland of the rabbit, the lachrymal gland of the rabbit, and added very considerably to the knowledge accumulated by previous observers.

In a mucous gland (submaxillary gland of the dog) the cell characters vary according to the state of activity of the gland. If the gland has not been actively secreting for some time the cells are in a condition which is known as "loaded" or "charged." If the gland has been actively secreting the condition of the cells is different. They are then known as "unloaded" or "discharged." In the loaded or resting

state the cell, in fixed preparations, is transparent and stains faintly. The nucleus is seen near the basement membrane. A resting cell is then filled with mucinogen.

After a period of active secretion the cell is found to be smaller, giving rise to a wider lumen in the alveolus. The cell contains a variable amount of stainable material. The nucleus occupies a more central position. The transparent non-staining mucinogen has been largely replaced by ordinary staining protoplasm.

In the "serous" glands and the pancreas, the changes in the histological appearances during different phases of activity are marked by the disposition of definite, discrete granules. In a "loaded" cell the granules are abundant and nearly fill the cell, leaving only a narrow clear zone next to the basement membrane. The lumen is often scarcely visible. The nucleus is hidden. In a "discharged" cell the granules have for the most part disappeared, and those that remain are found only next the lumen. There are now two distinct zones in the cell, a narrow inner granular zone and a clear transparent outer zone, the relative width of the two depending on the degree of activity of the gland. The cells are smaller and their outlines more distinct; and so the contour of the alveolus is altered. In the well-known figure taken from Kühne and Lea the discharged alveolus shows distinct indentations at the junction of two cells, while this is not seen in the case of the loaded gland. The lumen in the discharged alveolus is wide.

It is clear that during the act of secretion the cell granules are emptied out into the lumen of the alveolus.

The granules of secreting cells differ in size and in chemical reactions in different glands. Thus in the mucous glands they are large and conspicuous. In serous glands they are small, while in the pepsin glands they are small erstill. The granules are probably liquid or semi-liquid. The nucleus has been supposed to play an important part in the formation of the secretion products. It appears to give out a portion of its chromatin which may form the basis from which the granules are evolved. The separated portion may remain for a time, forming the *paranucleus* (*vide supra* p. 20). This was first described by Gaule and Nussbaum. It appears from

recent work that the structure in question really represents an aggregation of mitochondria.

The nucleus becomes larger during the activity of the secreting cell. This simply means that there is increased metabolic activity, and is an expression of the general function of a cell-nucleus. There is during secretion no tendency towards cell-division, and the cell is not bodily destroyed in secretion.

But the salivary glands and the pancreas are not the only glands in which there is histological evidence of a secretory process. The "central" or "chief" cells of the gastric glands also show changes. In the newt these were studied many years ago. When the stomach is at rest the cells are loaded with granules. During digestion these granules disappear or are represented only by a zone next the lumen. Similar changes in mammalian gastric glands have been described, and in more recent years numerous instances have been cited where it is possible to observe changes, more or less distinct, in various kinds of secreting cells. We shall have occasion to refer to this matter again when discussing the question of secretion in certain of the ductless glands.

The "all or none" principle first applied by Bowditch in 1871 to the frog's heart has since been extended to other kinds of muscle and to nerve. Bayliss (12) and Metzner have pointed out, as a necessary corollary of this, that glands which receive their normal stimuli through the nervous system, should show the same phenomenon. Such is actually alleged to be the case with the chromaphil cells of the adrenal body, the submaxillary serous glands, and the glandular portion of the pituitary body. The "refractory period" first demonstrated in the frog's heart by Marey in 1885 has been found to apply to skeletal muscle, to nerve, and to sense organs, and was considered by Gotch to be a general phenomenon of living substance.

It seems probable that these considerations account for the fact that each organ has a greater amount of substance than is necessary to carry out its function during normal conditions. Boycott has found that this "reserve force" is present not only in the normal state but also in atrophy and hypertrophy. The units of an organ are not all in a state of activity at times when the organ is carrying out a moderate amount of work. Those which are active are doing as much work as they are capable of. The total activity of an organ varies with the number of units which are active, and each period of activity is followed by a refractory period and an interval of rest. The units take duty by rotation. In glands the unit may be a cell, an acinus, a group of acini, or even a part of a cell. In the liver the zones of the lobules are the probable units. In the kidney it is possible that each glomerulo-tubal system is a unit (Khanolkar). There is experimental evidence to show that the various units of which the kidney is composed are not all active during moderate activity of the organ. This focal activity may possibly be determined by the tonus of the capillaries described by Krogh in 1920 (Khanolkar).

As we shall see, two different kinds of cells are commonly found in the "ductless glands," and if we are satisfied that a process of secretion is actually occurring in these organs, it is tempting to suppose that the two varieties of element represent two different phases of secretory activity.

CHAPTER III

THE PHYSICAL AND CHEMICAL MECHANISMS OF SECRETION

A. Introduction

We have seen that in a great variety of secreting glands it is possible to follow by histological examination the different phases of the secretory process. Concomitantly with the flow of secretion from the ducts of the gland there are definite changes in the secretory granules within the cells, which changes can be watched with comparative ease. We have next to regard the process of secretion from the standpoint of physical chemistry in order to see in how far the known facts of this science will account for the happenings in secreting structures. It is not possible at the present time to bridge over the gulf between the two series of observations, except possibly in respect of a few isolated cases where microchemical methods have been of service.

The term secretion is not used in a very definite or restricted sense, but has been applied and is still applied to several different processes. In all living things, animal and vegetable, different substances are produced by the metabolic activity of the living protoplasm. In uni-cellular organisms the substances so produced may either be of some use in the cell itself as, for example, to help in the digestion of food material ingested by the organism, or the substances may be of the nature of waste products and are cast out of the cell into the surrounding medium. In higher (multicellular) animals again the substances produced may be of service in the cell itself. They may, however, be destined to be utilized in some other part of the body. They may, for example, help in the digestive functions or they may serve the needs of

the whole body. Or, once again, they may be of no use to the body, and so are cast out as waste products.

So that, according to a usage which is tolerably general, the term "secretion," in the broadest sense of the word, means the separation out of substances from or by the agency of the living protoplasm. But the conception has been made to include also the preliminary preparation or a more or less complete elaboration of the materials which are supplied by the blood.

Johannes Müller used the term "secretion" to apply to the manufacture or preparation of the substances, while their elimination from the gland was called the "excretion." The term "excretion" has also by some authors been applied to the cases where substances, already present in the blood, were simply separated out by the glandular cells and poured forth in the secretion, as in the case of urea and the kidney. But later usage has generally made the term "excretion" apply simply and solely to the waste products and their processes of elimination. Again, excretions may be and frequently are got rid of by means of processes which are recognized as being of a secretory nature. The kidney is considered by some observers to "excrete" by means of actions which are true secretions. In the discussions on this point it is usually conceded that one of the chief criteria of secretion is the occurrence of a definite chemical synthesis. It would not be safe to urge this point too strongly. A glandular structure may be conceived to be capable of manufacturing useful chemical substances by a cleavage or breaking down process.

The secretions are the vehicle for such materials as enzymes.

It is, however, necessary to point out that (in the past more than at present) many kinds of tissues have been referred to as secretions. Thus the skeletal tissues of invertebrates such as the calcareous shells of the Foraminifera, the chitinous case of insects, cell membranes, etc., are frequently called secretions. Sometimes certain intercellular substances such as are found in the fibrillar connective tissue, cartilage, and bone are reckoned among the secretions. In spite of this it will be well where definite morphological structures are formed not to employ the term secretion. Thus the ova

and spermatozoa are not properly speaking secretions, though of course the seminal fluid as a whole is a secretion or rather a mixture of secretions. It follows that the organs which give rise to definite structures are not strictly speaking glands. Minot calls the ovary, testis, lymph glands, red-marrow, etc., *cytogenic glands.*

The cells of a gland produce substances which are not present in the blood supplied to them, and water passes from the blood into the gland and out with the secretion. The object of the secretion of water is clearly to carry out substances in solution. Besides the water we find in the secretions other substances derived from the blood, such as diffusible salts, notably sodium chloride.

As we have already seen, we apply the name "excretion" to such activity as that of the kidney in which certain metabolic products as, for example, urea, which are injurious to the animal are separated from the blood and cast out.

Nothing will be said for the present about the process known as "internal secretion." This will be dealt with in a later chapter, and we shall see that it is in many important respects something very different from secretion in the sense in which we are now using the word.

B. The Secretion of Water

We are still far from an understanding of the actual mechanism of secretion. And this must be so, until we know more of the chemistry of the living cell and its changes in varying states of rest and activity. The passage of water from the blood into the secretion is perhaps the most important of the points to be discussed.* It is at any rate the phenomenon which is most general in all the different varieties of secretory processes.

If the blood-pressure were greater than the osmotic pressure of the blood and if the membrane between the blood-vessels and the lumen of the duct could be regarded as a semi-permeable membrane, then we could see without

* I have made considerable use in writing this chapter of the account of secretion given by Bayliss,[12] but I have referred to the original papers in all cases where this was possible.

difficulty that pure water would be forced through. But such is not the case. The osmotic pressure of the blood may be 5,000 mm. of mercury, while the blood-pressure is rarely more than about 200. But the secretion is never pure water, so that the difference of pressures is not so great as just stated. If the membrane is permeable to the crystalloids but not to the colloids of the blood, a much lower blood-pressure will be able to filter through a solution containing all the crystalloid constituents of the blood in the same concentration as in it (Bayliss). This is probably what happens in the case of the kidney (glomerular secretion). It must be remembered that in this case there is a constant difference of pressure on the two sides of the membrane and that this is more than sufficient to push through a protein-free fluid from the blood into the ducts. Certain crystalloids adsorbed on the colloids may be held back by the latter (Bayliss). Such a membrane may be imitated by Martin's gelatine filter.

The attempts to explain the passage of water through living membranes were instigated by the epoch-making researches of Pfeffer in 1877, which led also to van't Hoff's theory of solutions. In 1906 Lepeschkin [86] employed "Pfeffer's 1st schema" to account for the secretion of drops of water at the tips of the aerial hyphæ of the fungus *Pilobolus*, as well as at the apex of the "hydathodes" of various plants.* The scheme supposes a tube filled with a solution of a certain concentration and closed at one end by a membrane impermeable to the solute and at the other by a membrane permeable to it. If the tube is immersed in water there will be a flow of liquid from the permeable end as long as any osmotically active substance is left in the tube. As the result of experiments on the structures above mentioned Lepeschkin concludes that the secretion of water takes place in accordance with "Pfeffer's 1st schema," that is to say, an unequal permeability for dissolved substances in the membranes at the opposite poles of the cells.

How far similar processes may be assumed to occur in the secreting glands of animals is not certain, but if we can assume that the secreting cells have a membrane on the

* In the majority of instances, however, the hydathodes are "pressure hydathodes," and the fluid is squeezed out through actual apertures.

ends next to the blood-vessels which is impermeable to some substances produced in the cells, while on the ends next to the lumen the membrane is permeable to these substances, we can account for a flow of water as long as these osmotically active substances are being formed. They are carried out with the secretion through the membrane permeable to them (Bayliss).

It seems not out of the question that the same thing might sometimes be achieved by a process of simple diffusion. In cases where such a diffusion could occur, the hypothesis would afford a simpler explanation of the facts.

It is clear that any such mechanism can only be effective where the secretion has an osmotic pressure less than that of the blood, as in the cases of the saliva, sweat, milk and bile. In the kidney, it is now very generally supposed that the filtrate from the glomerulus becomes more concentrated as it passes along the tubules.

There are many arguments in favour of the view that secretion and absorption employ fundamentally the same mechanisms. If our model tube provided with membranes of unequal permeability be reversed in position it would represent the processes of absorption and not secretion. We may suppose that some such reversal has occurred in the cells which normally absorb water, such as those of the intestine.

The discovery of Ludwig in 1851 that the pressure under which the secretion of the submaxillary gland occurs is greater than that of the blood in the carotid artery rendered the hypothesis of a simple filtration untenable. The difference of pressure shows that work is done in the production of the secretion. The result may be due to the manufacture of osmotically active substances in the cells and to the presence of an impermeable membrane next the blood-vessels (see Fig. 3).

In the case of the kidney as we have seen, where the osmotic pressure of the secretion is greater than that of the blood, any osmotic explanation is difficult and the hypothesis of "protoplasmic activities" has to be put forward to cover our ignorance. But after all it is only a matter of degree, for even in the simplest cases we have to assume the continued production of osmotically active substances. This

must involve the splitting up of large colloidal particles into smaller molecules. When a gland is made to secrete it is sometimes possible to observe a solution of the zymogen granules, and it is possible that this is a histological expression of the process in question.

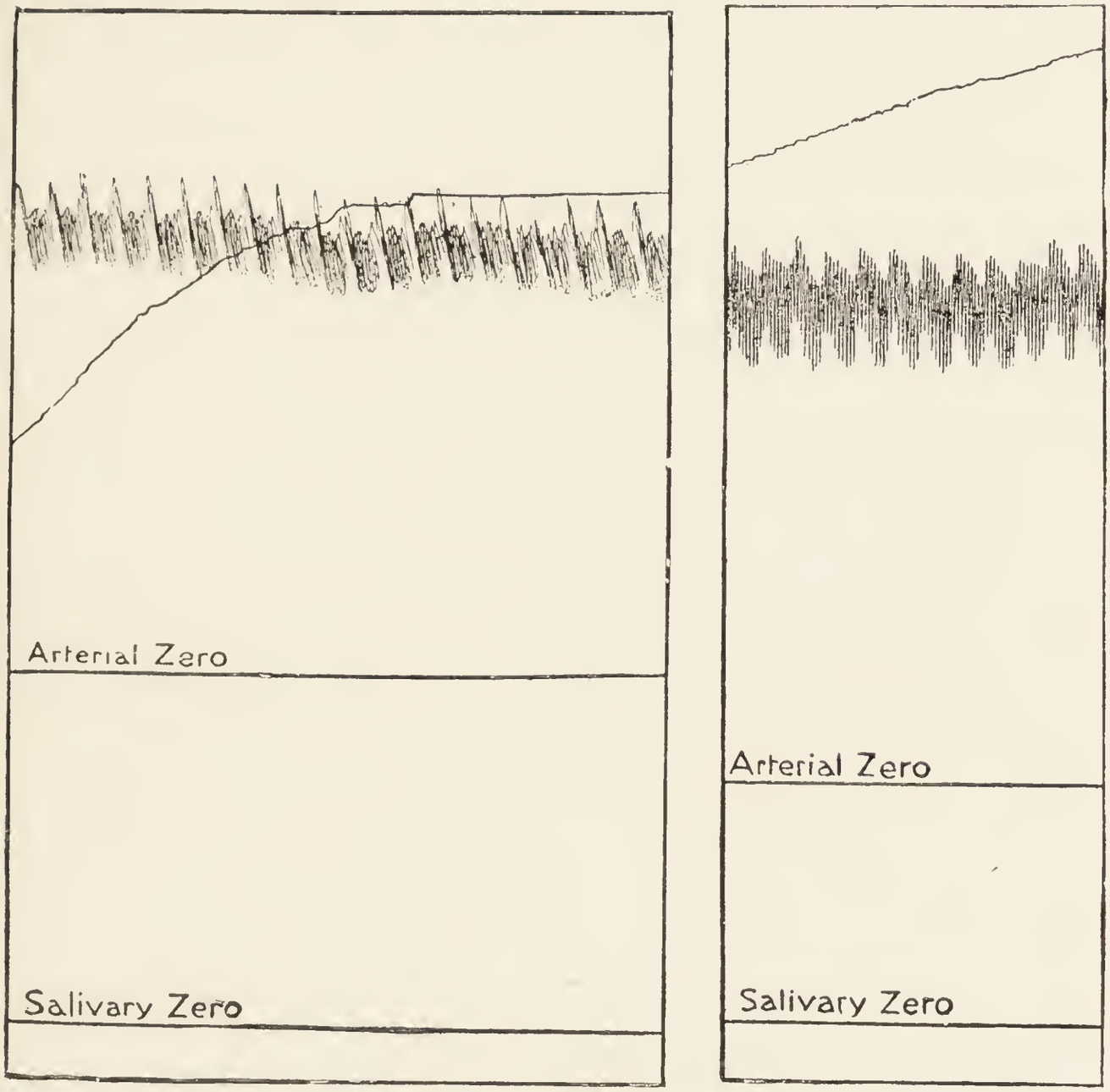

FIG. 3.—Tracings from two different Dogs showing the Arterial (Carotid) Pressure and the Secretory Pressures of Saliva during Excitation of Chorda tympani Nerve. (Hill & Flack, from *Proc. Roy. Soc.*)

There is good reason for believing that there is a *change in the permeability* of the cell during the process of secretion. Garmus,[56] using the vital staining method introduced by Ehrlich and employing the mucous glands in the nictitating membrane of the frog, made a careful study of the reactions of the cells to various stains under different physiological

conditions. He succeeded in staining these cells with methylene-blue, neutral red, toluidin-blue and other dyes. In some cases discrete granules became stained, while in others the stain was diffuse.* The intensity and duration of the vital staining is influenced by pilocarpine and atropine. After treatment with pilocarpine, which excites secreting cells, the vital staining of the gland cells comes on more quickly and is more intense than in normal glands. After the administration of atropine, which checks the activity of secreting cells, the vital staining is fainter and appears more slowly than in the normal condition. So that the vital staining of gland cells is dependent on their physiological condition. Garmus distinguishes between the " static " and the " dynamic " permeability of the gland cells. The same condition which determines an increased absorption of water and salts, induces also an increased permeability for stains.

A similar increase of permeability of the cell membrane of the sweat glands has been described. This occurred after stimulation of the nerves to the gland, and was shown by a diminution of the galvanic polarization of the cell due to the increase of the permeability to ions.

The influence of *surface tension* in amœboid, and other movements has been suggested by several authors for the last fifty years. In 1910 Macallum suggested that surface tension is of great importance in influencing the distribution of salts in living matter. Surface-tension is a particular case of the molecular attraction between the molecules of a fluid. On the surface of the fluid the molecular attraction manifests itself as surface-tension. In the interior each molecule is affected in all directions equally by the molecular forces exerted by the surrounding molecules. The molecules of the superficial layer, on the other hand, are affected only by their own forces and by those of the molecules beneath them ; the attractive force they would exert externally is thus free, and so the superficial molecular layer forms an elastic membrane.†

* Garmus says that there is no relation between the solubility of a dye in lipoids and its power of vital staining (against Overton).

† See Brinkman and Györgi, " Contraction of a Drop of Water by Change of Reaction." *Proc. Physiol. Soc.*, Oxford, July 7, 1923.

Macallum's views are based upon the Gibbs-Thompson law "that when a substance in solution increases the surface tension of a fluid system (e.g. a drop of water) it is less concentrated in the surface layer than in the rest of the system, while a substance that lowers the surface tension of the system is more concentrated in the surface film than it is in the rest of the system."

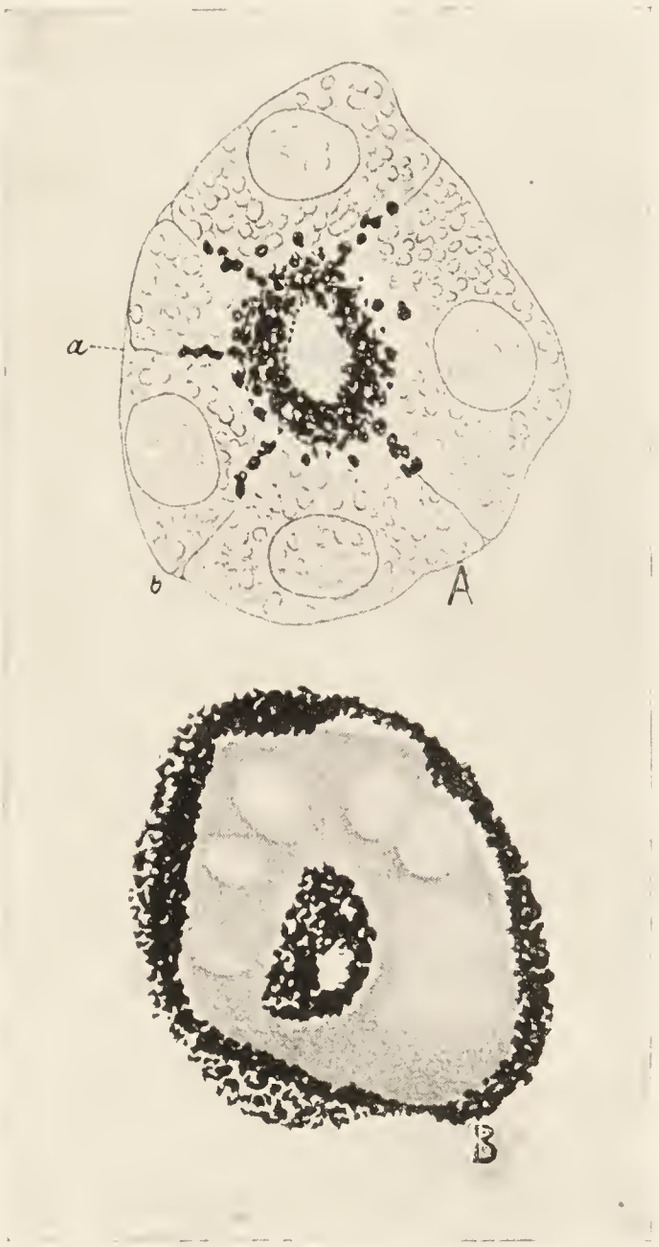

FIG. 4 (*A* and *B*).—A. Cross-section of an Acinus of the Pancreas of a Guinea-pig showing Surface Condensation of Potassium on the Lumen Border of the Cell.

B. Cross-section of convoluted Renal Tube in Dog showing Surface Condensation outside the Tubules and on the Wall of each Renal Cell adjacent to the Lumen.

The distribution of potassium as revealed by the cobalt hexanitrite method is represented in black shading. (Macallum.)

Macallum studied by microchemical methods the distribution of potassium salts in living cells. He found that the hexanitrite of cobalt and sodium, when employed in a certain concentration in a solution of sodium nitrite and acetic acid, constitutes a delicate test for potassium, and that the test can be carried out in the interior of animal and vegetable cells. In the pancreas of the rabbit and guinea-pig an extraordinary condensation of potassium salts was found in the cytoplasm of each cell adjacent to the lumen of the tubule. During all phases of activity the potassium salts are confined to these surfaces inside the cell. "The low tension at the lumen border and the high tension at the base of the cell cannot but be a factor in promoting the processes of secretion." Theoretically, in an active gland there

must be at least three different values for surface tension, viz. (1) Cell-lymph interface, on the outer face, through which substances enter the cell from the blood. (2) Cell-cell interface where the cell-wall is in contact with none of the other cells of the gland. (3) Cell-lumen interface, through which the water and secreted substances pass out. Macallum found that during activity there was the densest condensation of potassium at (3) the cell-lumen interface, less at the cell-cell interface and least at (1) the cell-lymph interface. According to the Gibbs-Thompson law, Macallum interprets these results as meaning that during rest there is no marked difference of surface tension at the gland interfaces, while during activity a high tension develops at the surface between cell and lymph, a low tension between cell and lumen, and that the cell-cell interface is intermediate in this respect between the other two. Macallum records the interesting observation that the intestinal cells, engaged in absorption, leave the accumulation of potassium salts at the end of the cell opposite to the lumen. Thus there may be no fundamental difference between absorption and secretion. The two functions would be expressed as due to the capacity which the cells have of maintaining differences of surface tension on their two active cell surfaces.

It seems very probable that the observations of Macallum may help to explain the passages of substances from the cell-body to the lumen, and so the changes in surface tension may assist the processes of osmosis and diffusion.

Macallum believes that in the attempts to "explain" the phenomena of living things one aspect of molecular physics (the Arrhenius theory of dissociation and van t' Hoff's gas theory of solutions) has been unduly emphasized, while others have been neglected. One of these is surface tension. The others are the intrinsic pressure of liquids, viscosity, and the thermodynamic relations of solutions (Macallum,[90] Bayliss [13]).*

* The work of Macallum has not escaped criticism, but there can be no doubt that he has done a useful service by calling special attention to a possible factor in vital processes which had been neglected by previous workers.

C. The Work done in Secretion

It has not been possible to detect by direct measurement any formation of heat in glands. The gland is thus a very efficient machine, most if not all of the additional energy set free during the act of secretion being used in the actual processes of secretion, in fact in doing *chemical work*. The chemical processes concerned in the manufacture of specific products are so complicated and so little understood that we must be content with the indirect method of studying the oxygen consumption or the carbon dioxide output, or the using up of sugar in the blood passing through it.

Barcroft and his co-workers have devoted special attention to the oxygen consumption of glands during rest and activity. This is done by estimating the oxygen content of the blood as it goes to the gland and the oxygen content of the blood in the vein leaving the gland, as well as the amount of blood passing through the organ during the time of the experiment. An increase in the blood-flow does not lead to increased oxygen-consumption (Barcroft and Müller [9]) though increased glandular activity, as, for example, after stimulation of the chorda tympani, does so to a marked degree. In the cat the oxygen used by the submaxillary gland in a resting state under experimental conditions is 0·016–0·028 c.c. per gram per minute. This amount may be five times as great during activity. In the case of chorda stimulation the period of increased oxygen outlasts that of salivary secretion by several minutes (Barcroft and Piper [10]), the disparity being greater the less "fit" the gland. It seems very likely that glandular tissue, like muscle, is a mechanism in which the oxidation serves to replenish a store of potential energy which is liberated in the act of secretion (see Fig. 5).

The blood-sugar changes in secretory glands have been studied by several observers. Anrep and Cannan [2] find that the resting submaxillary gland consumes blood-sugar to the amount of 0·8–2·9 mgms. per gram of gland per hour. Atropine does not change the blood-sugar consumption of the resting gland, but pilocarpine increases it in proportion to the rate of secretion. The average figure of blood-sugar consumption per 1 c.c. of saliva secreted was 1·5 mgms. per

gram of gland per hour. Anrep and Cannan compare their figures with those of Barcroft for the oxygen consumption of the resting gland. As we have already seen, the latter observer recorded on an average 1·2 c.c. of O per gram per hour. Assuming the substance oxidized to be glucose, this would imply a consumption of 1·8 mgms. of glucose—a figure which agrees fairly well with that given above.*

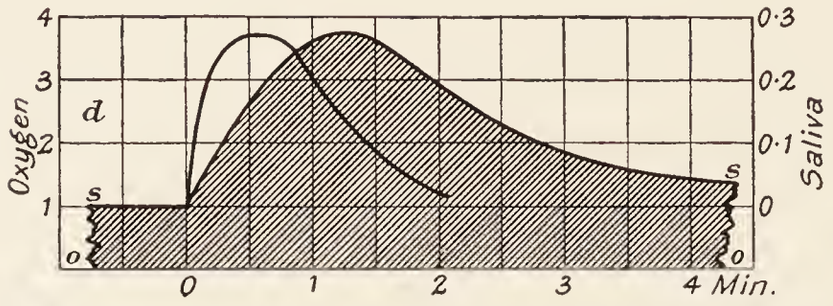

FIG. 5.—Oxygen Consumption of Resting as compared with Active Salivary Gland.

Line represents mean rate of salivary secretion in c.cs. per minute in three experiments. S—S represents base line for saliva. O—O = ogygen base line. Black area = oxygen used by the active as compared with the resting gland. (Barcroft and Piper.)

A method of calculating the osmotic work of glands is given by Bayliss. [12]

D. The Nervous and other Mechanisms of the Excitation of Secretion

The mechanism of excitation, our knowledge of which is based upon the surest foundations, is that by which glands are excited to perform their function by means of reflex action. We have to consider in these cases what are the afferent nerves, the efferent nerves, and the "centres" in the central nervous system. Most of our knowledge in this direction has been derived from a study of the salivary glands.

1. *Submaxillary Gland of the Dog and Cat*

The secretory nerves to the salivary glands were discovered by Ludwig in 1851. He found that stimulation of the chorda tympani nerve causes a flow of saliva from the submaxillary gland. We know now (owing chiefly to the work of Heidenhain [69] and Langley [81]) that this gland has two separate nerve supplies—the chorda tympani and the sympathetic. It is also well known that the character of the secretion

* Note that Barcroft's amounts are given for the minute, those of Anrep for the hour.

produced varies with the nerve stimulated. Stimulation of the chorda gives rise to a copious watery secretion, while excitation of the sympathetic produces a scanty viscid saliva. Heidenhain thought that we have to deal with two different kinds of fibres, one set to look after the water and dissolved salts, the other being concerned with the production of the specific solid constituents of the secretion. Heidenhain called the first set "secretory," the second "trophic," and he considered that the latter cause chemical changes in the cells leading on the one hand to the growth of protoplasm, and, on the other, to the conversion of the stored-up material into a more soluble form. It was supposed that the proportion of these two kinds of fibre is different in the cranial and sympathetic nerves. The cranial nerve has a majority of secretory, the sympathetic of trophic fibres. The growth of protoplasm was deduced by Heidenhain from an increase in carmine-staining material in the cells.

Langley, on the other hand, points out that in the cat the chorda and sympathetic nerves both produce the same kind of saliva. Since the sympathetic contains constrictor fibres for the vessels, while the chorda contains dilator fibres, Langley holds that the restricted blood-supply accounts for the thick viscid saliva produced by the sympathetic; and that there is only one kind of nerve-fibre running to the gland-cells.

But it is found that in a dog with a permanent submaxillary fistula a free flow of saliva may be brought about by putting into the mouth powdered meat or 0·25 per cent. hydrochloric acid. The amount of saliva poured out may be the same in both cases, but that secreted after the giving of powdered meat contains more than twice the amount of solid matter as that after the hydrochloric acid. The blood-flow through the gland is accelerated to an equal degree by both kinds of treatment. Removal of the superior cervical ganglion does not make any difference to the result, so that the chorda tympani must contain "trophic" fibres in Heidenhain's sense. This might be expressed according to Heidenhain's theory by saying that acid excites the "secretory" and vaso-dilator fibres, while the trophic fibres are very little affected. Meat excites both the secretory and the trophic

fibres of both nerves, as well as the vaso-dilator fibres of the chorda tympani (Babkin [6]). But Babkin thinks that the same nerve, the chorda tympani, may be affected through the nerve centre in different ways, qualitatively and quantitatively. Langley has since suggested that different parts of the centre may be excited by the different kinds of impulse. It is clear that the machinery exists for two different kinds of secretion, varying with the nature of the reflex stimulus; but it will be seen from the foregoing account that it is not yet known what is the precise nature of this machinery.

Normally, of course, the secretion of saliva is brought about by reflex action. The afferent nerves are the fifth and the glossopharyngeal supplying the mucous membrane of the mouth. The efferent fibres have been already discussed.

Artificial perfusion of the submaxillary gland has been carried out by Demoor [39 40 41 42] and has given some very interesting results. The cells of the gland seem to be very sensitive to the osmotic pressure of the fluid perfused. The rapidity of flow varies with the content of the fluid in salts. A hypertonic solution passes through more rapidly than an isotonic, and an isotonic more rapidly than a hypotonic. When Locke's fluid (isotonic) is being perfused, even with proper oxygenation, excitation of the chorda tympani induces vaso-dilatation, but no secretion of saliva. But if a small quantity of the animal's own serum be added to the perfusing fluid, then stimulation of the chorda is followed by secretion. Reflex secretion follows the same rule.

It is interesting to note that the reaction to pilocarpine occurs with Locke's solution only. Demoor thinks that this indicates that the response to pilocarpine is not a true secretion. We have already seen that the drug causes very profound changes in the secretory cells.

If the serum of another species of animal be added to the perfusing fluid, the stimulation of the chorda only produces a very slight effect.

2. *Parotid Gland*

The parotid gland is also provided with nerve fibres from two different sources. One set passes along the glosso-

pharyngeal and its tympanic branch (Jacobson's nerve) to the tympanic plexus. From this fibres pass along the small superficial petrosal nerve to the otic ganglion, and thence by the auriculo-temporal to the gland. The other set belongs to the sympathetic and consists of fine fibres running along the walls of the arteries.

As in the case of the submaxillary glands a different result is obtained on stimulation of the different nerves. The cranial fibres cause a copious secretion of a fluid poor in solids. As a rule there is no secretion on stimulation of the sympathetic, probably because the thick fluid blocks up the duct.

Demoor also finds that the addition of saliva to the perfusing fluid causes the gland to secrete. He finds further that there are two exciting substances in the saliva, one destroyed at 60°C. which has the power of initiating a flow of secretion, and another (not destroyed at this temperature), which can increase the rate of flow when this has nearly stopped (subsequent to stimulation of the chorda tympani). As a result of these findings Demoor suggests that when the nerve causes secretion it produces a substance which acts on the secreting cell. He suggests that this substance is of the nature of a hormone.

Lovatt Evans [48] describes an increased amylo-clastic action of the saliva after ingestion of carbohydrates. Chewing without swallowing does not bring about the increased production of the enzyme. This appears to be an instance of adaptation, in which there is an increased production of an appropriate enzyme in response to the stimulus of a particular article of diet. He thinks that a chemical substance of the nature of a "hormone" is produced by the action of the carbohydrate on the mucous membrane of the stomach. Other instances of adaptation have been alleged.

In general it may be stated that the amount of lymph-flow from an organ is increased by activity of the organ. According to Starling this may be explained by the increased osmotic pressure in the fluid of the lymph spaces brought about by the production of a larger number of small molecules from larger ones during active metabolism. The lymph-flow of the submaxillary gland is increased by chorda stimulation, but not after the administration of atropine. This

indicates that the increased lymph-flow is due to the activity of the gland cells.

The same kind of discussion in respect of "secretory" and "trophic" fibres applies both to the submaxillary and the parotid glands.

It is worthy of note that the saliva of the dog does not contain any enzymes.

A salivary gland may give out during twenty-four hours a quantity of secretion weighing ten or twelve times as much as the gland itself. Heidenhain noted that the strength of the stimulus determines both the rate of secretion and the concentration of the saliva. With a strong stimulation of the chorda tympani the amount of saliva was increased four-fold, while the percentage of organic substances was more than doubled. The percentage of salts was also increased.

Claude Bernard [20] found that when the chorda tympani in the dog was cut the submaxillary gland began after a few days to pour out a slow continuous secretion, which continued for some weeks, during which time the gland became smaller and smaller. It has been supposed that there are special fibres in the chorda tympani, whose function is to inhibit the activity of the gland. When these are cut the secretion goes on spontaneously. The evidence for the existence of such special "inhibitory" fibres is not very strong.

Various drugs affect the secretion of saliva. Pilocarpine causes secretion of the salivary glands, as indeed of all the other glands. It is supposed that it acts directly on the gland cells and not through the nerve terminals. Adrenin, however, which also causes secretion of saliva in the cat, probably acts through the medium of the sympathetic nerve-endings. Atropine checks glandular secretion, and after administration of this drug pilocarpine will no longer call forth a secretion. Pilocarpine causes profound changes in the secretory cells.

3. *The Pancreas*

Claude Bernard was the first to observe that the flow of pancreatic juice depends on the passage of food into the duodenum, and it has been known for a long time that in a dog the flow of juice, although it begins immediately after

food has been taken, does not reach its maximum till two or three hours have elapsed. This is at a time when the greatest quantity of food is passing from the stomach into the duodenum. As to the precise causal relationship between the passage of food into the duodenum and the flow of pancreatic secretion, there is still much to be learnt.

M. Foster in his text-book of 1889 says : " Stimulation of the medulla oblongata or of the spinal cord will call forth secretion in a quiescent gland, or increase a secretion going on. From this we may infer the existence of a reflex mechanism, though we cannot as yet trace out satisfactorily the exact path of either the afferent or the efferent impulses ; all we can say is that the latter do not reach the pancreas by the vagus, since stimulation of the medulla is effective after the section of both vagi.

" A secretion already going on may be arrested by stimulation of the central end of the vagus, and the stoppage of the secretion which has been observed as occurring during and after vomiting is probably brought about in this way. This effect, which, however, is not confined to the vagus stimulation of other afferent nerves, such as the sciatic, producing the same effect, may be regarded (in the absence of any proof that the result is due to reflex constriction of the pancreatic and local vessels unduly checking the blood-supply) as an inhibition of a reflex mechanism at its centre in the medulla or in some other part of the central nervous system, much in the same way as fear inhibits at the central nervous system the secretion of saliva following food in the mouth. But if so, then we must regard the secretion of pancreatic juice as closely resembling that of saliva, inasmuch as it is called forth by a reflex act. Yet, it is stated that, unlike the case of saliva, the secretion of pancreatic juice continues after all the nerves going to the gland have been divided, an operation which would do away with the possibility of reflex action. Such an experiment, however, cannot be regarded as decisive, since it is almost impossible to be sure of dividing all the nerves."

We have seen in the case of the submaxillary gland, that a secretion (" paralytic ") will flow when the chorda has been cut, and it is conceivable that the same kind of thing might

happen in the pancreas. Moreover, as Foster says, it is almost impossible to be satisfied that all the nerves to the gland have been severed, unless very serious injury to the organ or its blood-supply be inflicted.

But in 1902 Bayliss and Starling [15] put forward their well-known theory of secretion by means of a "hormone"-*secretin*. Their view was that the acid of the gastric juice upon reaching the duodenum converts *prosecretin* manufactured by the epithelial cells into *secretin*; this secretin is then absorbed into the blood-stream, carried to the cells of the pancreas, and stimulates the organ to secretory activity.

The work of Bayliss and Starling was a sequel to that of Popielski and Wertheimer and Le Page, who had found that introduction of acid into the duodenum still excites pancreatic secretion after section of both vagi and both splanchnic nerves or destruction of the spinal cord, or even after complete extirpation of the solar plexus, and that secretion could also be induced by the injection of acid into the lower part of the small intestine. Secretion could be excited from a loop of jejunum entirely isolated from the duodenum.

Bayliss and Starling discovered that a flow of pancreatic juice could be brought about by taking an isolated loop of jejunum, dividing the mesenteric nerves supplying it, and then injecting acid into it. Further they discovered that a similar flow could be induced by cutting out the loop of jejunum, scraping off the mucous membrane, rubbing it up with sand and 0·4 per cent. HCl, and injecting the extract into a vein. They suggested the name "secretin" for the active substance, which, they suggested, is produced by a process of hydrolysis from a precursor "prosecretin" present in the intestinal cells.

For some years after the publication of this work, it was assumed by many writers that under normal conditions any nervous influence upon the flow of pancreatic juice is non-existent or at any rate negligible, and that the mechanism of excitation of the secretion is purely a chemical one. Bayliss and Starling, however, were careful to admit that their experiments did not disprove the existence of secretory fibres to the pancreas.

It has been known for some years from the work of Pavlov

and his pupils that if proper precautions be taken, stimulation of the vagus will call forth a secretion of pancreatic juice, even when the flow from the stomach is prevented. The spinal cord must be divided at the foramen magnum in order to eliminate a reflex inhibition brought about by the operation itself. Moreover, the flow from the gland may be inhibited by nervous influences, also through the vagus. Anrep finds that this "inhibition" of the secretion is accompanied by a dilatation of the gland, which is a result of a constriction

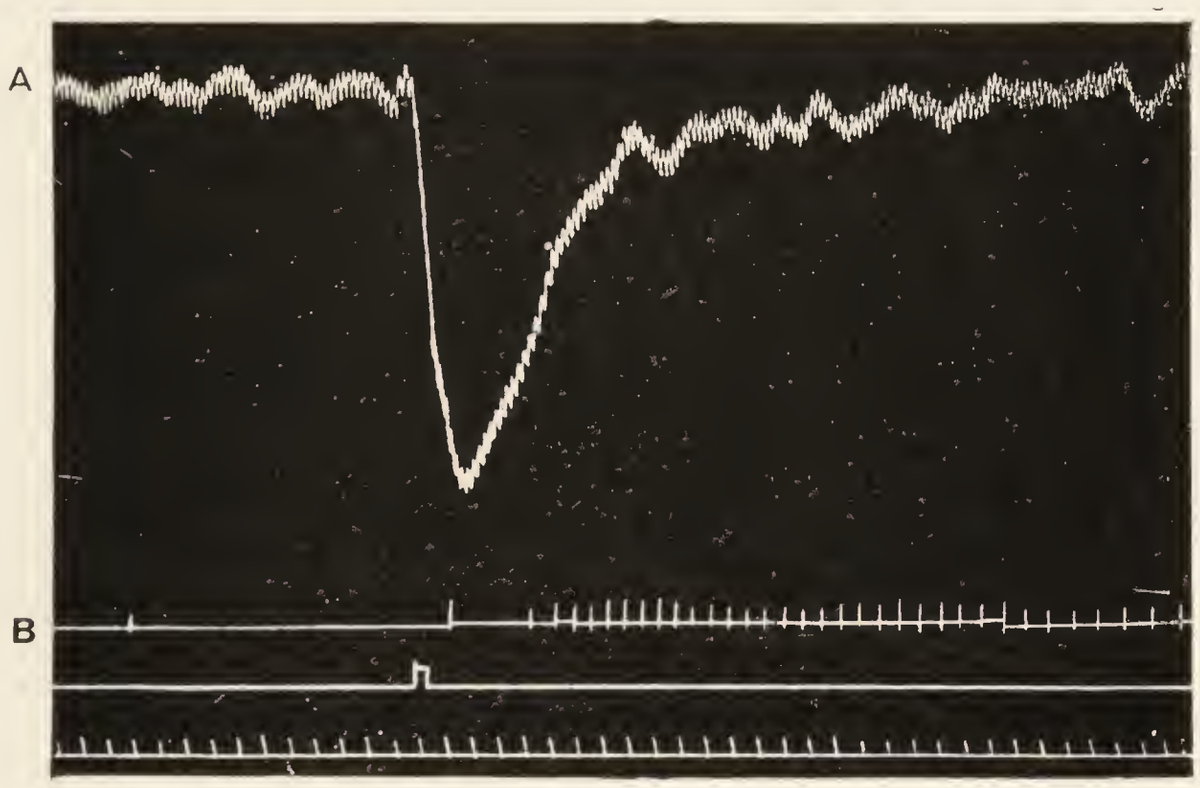

FIG. 6.—Action of Acid Extract of Mucous Membrane of Duodenum (Secretin) dehydrated by Alcohol.

A = Blood pressure. B = Drops of pancreatic juice. (Bayliss and Starling.)

of the pancreatic ducts or of a retention of the juice in the glandular cells which secrete it. If the peripheral end of the vagus be stimulated, the first stimulation is generally without effect. The next few stimulations usually give secretion, but only after a very long latent period.* With repetition of the stimulation the latent period gets steadily shorter, so that finally the flow of juice may start within a few seconds of the commencement of stimulation.

An important paper by Babkin, Rubashkin and Ssawitsch [7]

* There is a similarly long latent period when the gastric juice is secreted as a result of vagus stimulation (see p. 46).

deals with the two different kinds of secretion, that produced by the introduction of acid into the duodenum and that brought about by nerve stimulation. The best results are obtained by stimulation of the vagus, but similar though not so marked effects may be produced by stimulation of the sympathetic. The secretion by means of acid is characterized physiologically by the abundant pouring out of a fluid poor in protein and enzymes; and morphologically by slow and insignificant changes in the cell granules, by streams of fluid secretion in the cells and by the fact that the chemical nature of the secretion is unaltered as it passes into the ducts. The secretion by nerve stimulation is distinguished physio-

Fig. 7.—Alveoli of Dog's Pancreas—fasting, loaded with granules.

logically by the secretion of a small quantity of a thick juice rich in protein and enzymes, and morphologically by the extensive removal of zymogen granules from the cells and the signs of their elaboration. The juice in the ducts possesses different micro-chemical characters from the zymogen granules.

According to the above-named authors in acid secretion water flows abundantly through the cells and carries the granules with it into the ducts. Then the granules become dissolved and we find in the ducts a solution of a substance which is practically the same as that of the granules. The secretion is thus of a passive nature. The slight changes in the granules and the insignificant participation of the cell protoplasm in the secretory process may be correlated with the physiological facts—the poverty of the juice in

protein and its lack of enzyme activity (inactivity on coagulated white of egg with kinase in ten hours).

In nervous secretion the case is very different. The zymogen granules are elaborated in the cells and leave them in an altered state. The juice is here the result of active cell metabolism. The cells build up the granules, convert them into another chemical substance, and give to the juice a part of their protoplasm. Physiologically the juice is rich in protein and enzymes and has the power of digesting coagulated white of egg without the help of kinase in ten hours (see Figs. 7, 8, 9).

FIG. 8.—Alveoli of Dog's Pancreas after the Application of Acid to Duodenum.
Note slight disappearance of granules.

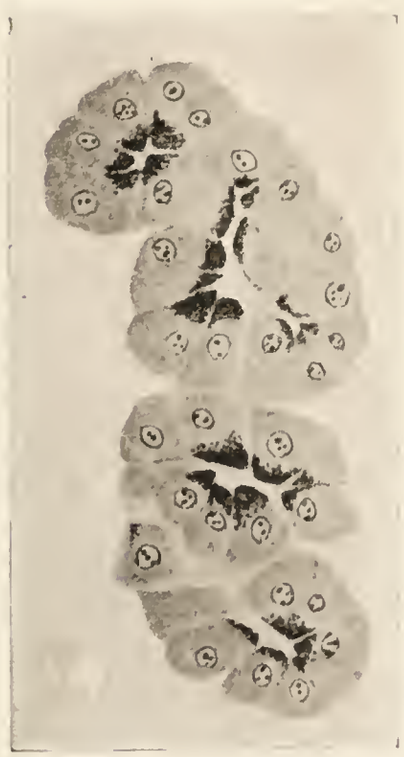

FIG. 9.—Alveoli of Dog's Pancreas after Vagus Stimulation.
Note almost complete disappearance of granules.
(Figs. 7, 8 and 9 from Babkin, Rubaschkin, and Ssawitsch.)

It is worth noting that fats and soaps give a juice rich in enzymes and solids.

Many writers are now inclined to believe that the normal mechanism of secretion of the pancreatic juice is a double one, the nervous, and the chemical. Others believe that it is purely nervous, while still others maintain that it is purely chemical. There is still another point of view, namely, that the chemical agent may act through nervous channels.

But it seems clear that the "acid" secretion is not the only kind of chemical mechanism to be considered. Not only acid, but water and oil, by their presence in the intestine will call forth a secretion of the pancreatic juice. Recent

work by Dodds and Bennett has shown that there is a fall of alveolar CO_2 pressure (below its original fasting level) in the later stages of digestion. It seems very probable that this must be due to the secretion of pancreatic alkali into the gut. Now this alveolar response does not differ materially with substances of varying reaction. Further, since perfect health can be maintained in cases of complete achlorhydria, it is justifiable to conclude that the formation of secretion by acid is not an essential factor in pancreatic secretin.

Hustin,[77] who apparently believes in a purely chemical mechanism, finds that perfusion of the pancreas with Locke's fluid, even if oxygen and secretin be present, does not give rise to a secretion. A more complicated series of factors is necessary. In addition to secretin, some substance derived from blood corpuscles must be present, as well as electrolytes. Hustin's work is interesting but not altogether convincing.

From the foregoing account it will be recognized that the information is not yet sufficient to warrant us in stating with any certainty what is the normal mechanism of the secretion of pancreatic juice.

4. *The Secretion of the Gastric Juice*

Pavlov and his co-workers have shown that if an animal with gastric and œsophageal fistulæ be fed, gastric juice will be secreted. So that the flow is clearly excited by the stimulus of the food in the mouth. But there is also a later secretion which seems to be connected with the presence of digesting food in the stomach. The psychical secretion, as well as that after a sham meal, is abolished by section of the vagi, and stimulation of the vagus when carried out under proper conditions will give rise to a flow of gastric juice. Normally, then, we may assume that the afferent channels for the reflex are the nerves from the mouth, or the nerves of sight, smell, or hearing. The efferent channel is the vagus.

The cause of the later secretion is not so clear. It is said to occur after the complete severance of the stomach from the central nervous system, and it is supposed by Popielski and others that we have to deal with a local nervous mechanism,

a reflex act, the whole arc being situated in the walls of the organ. Such peripheral reflex actions are not generally recognized, but it is possible that they have been unwarrantably dismissed from consideration. At any rate the "humoral" theory has not escaped very serious criticism. Edkins [45] performed a series of experiments which in his opinion pointed to the existence of a specific gastric secretogogue in the pyloric mucosa. The theory supposed that substances in the food or produced by the digestion of the food cause the pyloric secreting cells to manufacture a "gastrin" which is absorbed into the blood and then again reaches the glands by way of the circulating blood, and causes them to secrete. It is certainly true that 0·4 per cent. HCl extracts of pyloric mucous membrane when injected intravenously will cause a secretion of gastric juice. It has been shown, however, that an extract of many other organs and tissues will produce a similar result. But Edkins and Tweedy[46] report that when the pyloric and of the stomach was functionally separated from the fundus and different substances placed in the pyloric region the fundus responded by marked secretion. Ivy and Whitlow [78] have not been able to confirm this. They made a "two gastric pouch" preparation, and failed to obtain an increase in the secretion of the Pavlov pouch when various substances were applied to the mucous membrane of the pyloric pouch, which should have occurred according to the "gastrin theory." They also repeated the experiments (with slight modification) of Edkins and Tweedy, and found that the latter observers (by lack of controls) had overlooked the possibility of continuous secretion of gastric juice. Ivy and Whitlow found an increase of acidity in their experiments due to this continuous secretion.

Lim [87] has recently found that the blood of fed animals has no effect on gastric secretion when transfused directly or indirectly. Since there is no gastric exciting substance in the blood after meals he concludes that the excitant found in pyloric and other extracts is not secreted into the blood-stream and the mechanism of secretogogue action is not due to internal secretion.

Histamine, given by the mouth or hypodermically, is a very powerful excitant to the flow of gastric and pancreatic juices.

5. *The Secretion of the Succus Entericus*

The secretion of the small intestine obtained from an isolated loop by the Thiry-Vella fistula has little or no digestive action except upon the starches. It contains, however, *enterokinase*, an enzyme which activates the proteolytic enzyme of the pancreatic juice by converting the trypsinogen into trypsin, and *erepsin*, acting on proteoses and peptones, causing further hydrolysis.

It has been supposed that the mechanism of secretion is a chemical one—through the agency of a "secretin." The evidence on this point is not very clear. Pavlov believes the mechanism consists of two parts as in many other glands, the secretion of water and that of the enzymes. The former is, or may be, brought about by mechanical stimulation, the latter by a "specific excitation." Pavlov says that the most effective stimulus to the flow of mecus entericus is the presence of pancreatic juice in the loop of intestine.

6. *The Secretion of the Bile*

The liver is at first a compound tubular gland, but persists in this form only in the lower vertebrates. In higher animals it changes during embryonic life into a reticular gland by the union of its branched tubules. During this transformation the relation of the liver cells to the ducts becomes subordinated to their relation to the blood-vessels.

It is said that the liver cells have no cell wall. In their interior may be seen yellow pigment granules, fat granules, glycogen and finer granules of an unknown nature. Two nuclei are not uncommon.

The blood-supply to the lobules is well known and need not be described.

The earlier investigators carried out numerous experiments to discover a special nervous mechanism for the secretion of the bile. It must be remembered that "as an excretion the production of bile must be continuous and related, not to the processes of digestion, but to the intensity of destruction of the red corpuscles. On the other hand, bile as a digestive fluid is needed in the gut only during the period that digestion

is going on" (Starling). Bile is therefore stored in the gall-bladder between the periods of digestive activity.

It can be shown that stimulation of the spinal cord or the splanchnic nerve diminishes the flow of bile, while section of the splanchnics increases it. But these effects seem to be due entirely to the vasomotor effects. Increase of the blood-flow causes increased secretion, and *vice-versa*. Many writers are inclined to believe that the "secretin" which has been discussed in connection with pancreatic secretion, is of importance in adapting the secretion of bile to the period of digestion. It is certainly true that the injection of secretin into the circulation increases the flow of bile as well as that of the pancreatic juice. The effects of this as well as of other so-called cholagogues (with the exception of bile-salt) is, however, very slight. (Whipple.)

7. *General Observations on the Study of Secretory Mechanisms*

Many different modes of investigation have already been mentioned, and there are numerous sources of error in all of these. In animals under general anæsthesia we must always remember the serious influence of the anæsthetic itself. Not only is the temperature-regulating mechanism put out of gear by general anæsthesia, but there must be other profound changes in metabolism. Ether, for example, stimulates certain glands to secrete. The various drugs which are or may be used as adjuvants to the primary anæsthetic, or even as the principal or only anæsthetics, may any or all of them influence either the irritability of nerves or the activity of gland cells, or both of these. Thus morphine, curare, urethane, chloral and other drugs should all be used, if used at all, with due consideration of their pharmacological possibilities.

In stimulating nerves by the induced current, we must remember that the strength of the current, the frequency of interruption, and the duration and frequency of the periods of stimulation, all have their effect. Too strong a current may kill the nerve. Even under favourable conditions it frequently becomes necessary to change the position of the electrodes after a certain number of stimulations.

But electrical stimulation is not always the method best

calculated to give useful information. Experiments in which the natural methods of stimulation are imitated as closely as possible are always the best. It follows that reflex methods of excitation are more likely to lead to valuable results than direct influences on efferent nerves. Again, in studying the digestive glands, those experiments are the best where the natural stimulus of the food material is employed for experimental purposes. Chemical and mechanical means of excitation are also in many cases more reliable than the electrical.

E. Electrical Changes in Glands

Electrical changes occur in muscles, nerves, the retina, various plant tissues, as well as in secreting glands. The instruments used in recording these changes are galvanometers and electrometers. An account of the forms of apparatus used at the present time is given by Bayliss [12]. The string galvanometer and the capillary electrometer are those most generally employed. It is essential that the inertia of the moving parts must be as small as possible, so that rapid changes may be recorded without overshooting the correct position. They must either be aperiodic, but without more damping than just necessary, or their own vibration period must be shorter than that of any change to be measured (Bayliss).

It is customary to compensate the "current of rest" by sending in a small fraction of the current from a constant cell. In all cases non-polarizable electrodes must be used.

For a long time after the muscle currents were well known, it was thought that the gland muscles were the source of the electrical currents observed in glands. But gradually it was established that different kinds of glands in frogs and toads and the sweat glands in mammals give rise to electrical changes on excitation.

In 1885 Bayliss and Bradford [14] showed that the watery secretion of the submaxillary gland produced by stimulation of the chorda tympani nerve is accompanied by a deflection indicating that the hilum of the gland becomes positive to the surface; and that the viscid secretion produced by stimulation of the sympathetic, is accompanied by a deflection

of opposite sign. These observations lend strong support to the view that we have to deal with nerve fibres having two distinct functions (see above, pp. 37, 40). The sympathetic fibres require a larger dose of atropine to paralyse them than the chorda fibres do, and they require a larger dose to abolish the electrical changes due to their excitation. But there is evidence that in the chorda there are some fibres of the same nature as those in the sympathetic. Atropine when given in doses sufficient to paralyse the "secretory" fibres of the chorda still permits of an electrical deflection to be produced on stimulation. But now this is of the same sign as that from the sympathetic. This effect, normally, is swamped by the much larger opposite one, and is no doubt due to fibres of the same kind as those which preponderate in the sympathetic (Bayliss). That the results are not in any way due to vasomotor changes is proved by the observation that the electrical changes are abolished by atropine while the vascular ones are not. In the cat Langley had shown that both nerves give rise to a watery secretion, and Bayliss and Bradford found that the electrical change from both is of the same sign as that of the chorda in the dog, but is usually followed by one of the opposite sign. They therefore drew the conclusion that the electrical change of the sign of the typical chorda effect in the dog is due to the flow of water (together with salts of the blood) and that the other one is connected with the elaboration of the specific organic constituents of the saliva. In the parotid gland of the dog excitation of the tympanic plexus caused the surface of the gland to become negative to the hilum, and excitation of the sympathetic caused the surface to become positive to the hilum.

Bradford, some years later, found that when, as sometimes happens, the sympathetic gives rise to an abundant watery secretion, then we get a deflection of the kind usually obtained by stimulation of the chorda. The chorda effect is not due to mere flow of liquid along the ducts, for clamping of the duct had no effect on the electrical change. This must be due then to some processes in the secreting cells themselves.

Recent work by Gesell [58] shows that there is great variability of electrical deflections in glands. In the submaxillary

gland a prolonged chorda stimulation of about sixty seconds' duration usually produces a deflection of four crests, the result of a balance of four negative and four positive components. Variations may be observed due to changes in intensity and sequence of such processes as liberation of secretion, elaboration of secretion, recovery, etc. The contour

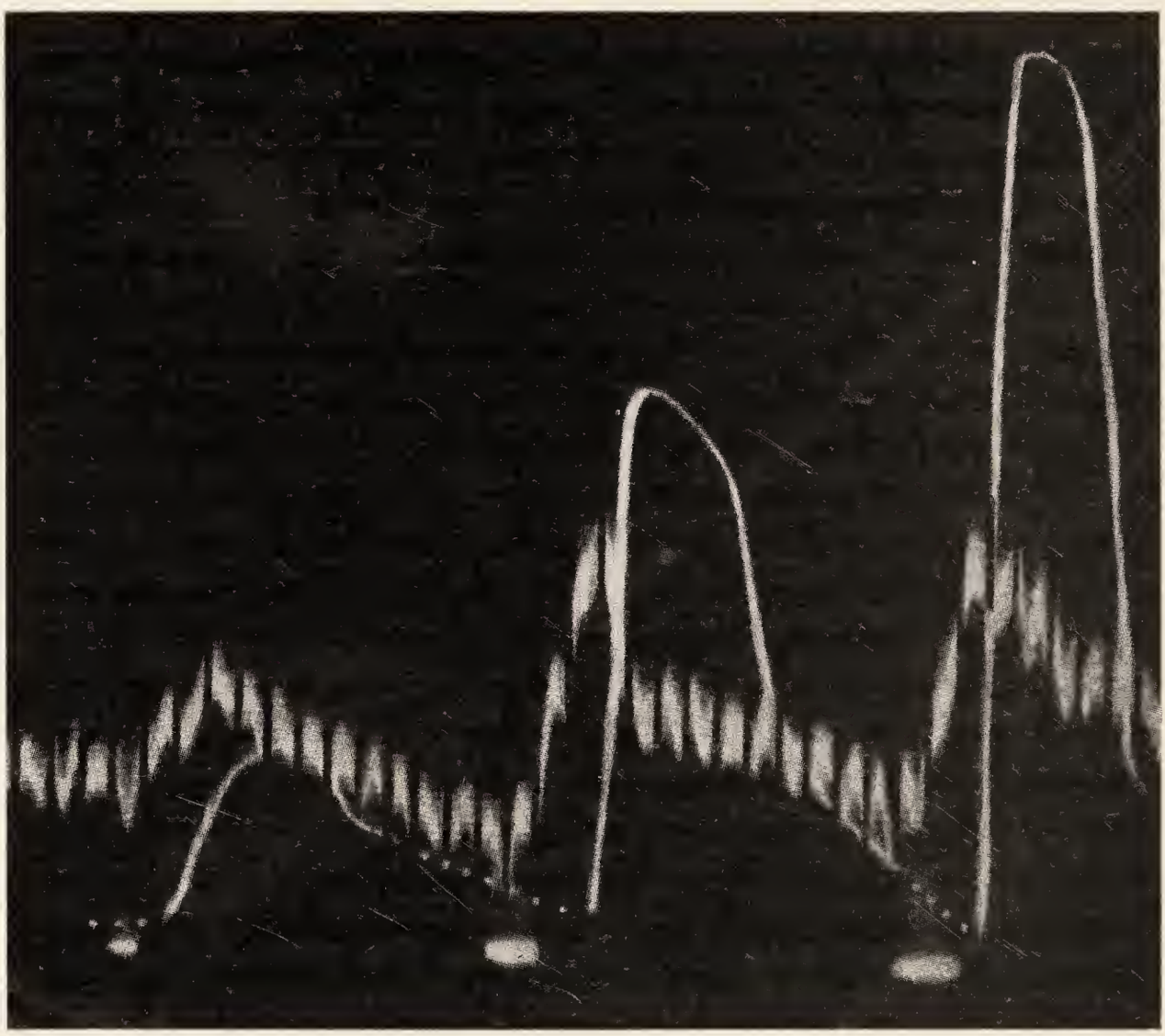

FIG. 10.—Three successive Injections of Secretin 2, 4 and 8 c.c. at Signals.

Broken curve = electrical variations; plain curve = rate of pancreatic secretion. Each interruption on electrical variations represents 25 secs. The smaller electrical deflections are due to intestinal movements. Broca galvanometer of 968 ohms. 1 millivolt = 600 mm. (From Anrep and Daly.)

of the electrical deflection may be altered by change of strength of stimulation and other factors.

The electrical variation of the pancreas has been investigated by Anrep and Daly [3]. These authors employed cats, and injection of secretin in a vein was the method used by them to bring about a secretion of the pancreatic juice. When the electrodes were placed upon the pancreas (one as near

as possible to the duct, the other on the splenic end) no deflection occurred on the injection of secretin into a vein, although a flow of juice occurred. When one of the electrodes was connected with the duct so that the secretion flowed over the electrode, injection of secretin into the vein caused a well marked deflection in which the duct was positive to the surface of the pancreas. The electrical variation always preceded the secretion, and was always observed when secretion was present. The mere flow of pancreatic secretion past the electrodes would not account for the electrical deflection. This was tested both by allowing ·9 per cent. NaCl to flow over the electrodes, and by closing the cannula distal to the electrode, when the electrical deflection occurred without any flow of secretion past the electrode. Again, the fall of blood-pressure brought about by injection of secretin is not the cause of the electrical variation, for independent alterations of the blood-pressure do not produce electrical changes.

The electrical changes in glands are not so strikingly useful in the study of secretion as the changes in nerve during conduction of an impulse, since in the latter case there is often no other method of showing the passage of impulses. But the electrical deflections of glands have already given some insight into the processes going on in the secreting cells, and the electrical method has been employed within recent years in order to demonstrate, in doubtful cases, that a process of secretion is really going on, as, e.g. in the case of the thyroid (*vide infra*).

The causes of the electrical deflections are not yet certain. We have seen that in secretion there is probably an increase in the permeability of the cell membrane at one end. There must be a separation of electrically charged ions and if there is a cell membrane which is permeable to one only of the oppositely charged ions it is not difficult to see in a general way how a difference of potential may be brought about.

CHAPTER IV

SOME SPECIAL FORMS OF SECRETION

A. The Secretion of Gases

In the gas-bladder of certain teleostean fishes are found peculiar glands whose function appears to be to secrete oxygen into the gas-bladder. It has often been assumed that though liquids or dissolved solids may be actively secreted, gases pass through living membranes by simple diffusion. It is now practically certain that a true gas secretion takes place in the gas-bladder of teleostean fishes.

The function of the bladder is to keep the specific gravity of the animal equal to that of the water at whatsoever depth. Oxygen is employed for the inflation (when the fish sinks and thereby experiences greater pressure) and deflation (when the fish rises and thereby experiences a diminution of pressure) of the bladder rendered necessary by the change of level. Two things may then be required, the secretion of oxygen and the getting rid of oxygen. In physostomous fishes, i.e. those with an open duct to the gas bladder, gas escapes through the duct and out through the animal's mouth. In physoclystous fishes (closed bladder) there is an oval area on the bladder wall where there is nothing but a thin layer of flattened cells between the interior of the bladder and a network of capillaries. This seems to be an arrangement for allowing oxygen to diffuse into the capillaries. This "oval" can be opened or closed by muscular action. So that deflation is clearly the function of the "oval." Inflation is, however, brought about by more elaborate structures—the "red bodies," "epithelial bodies," the gas or oxygen glands, and their associated *retia mirabilia.*

In the majority of physoclystous fishes the "red body" includes two structures, *the gas gland* and the *rete mirabile.* According to Woodland [124] the structure of the gas gland varies

in different species. The cells may be arranged to form a single unfolded layer, or the layer may be thrown into simple or complex folds, so that channels are formed leading from the surface of the glandular epithelium to the bladder lumen, or they may form a thick mass in which the deeper cells can only communicate with the bladder lumen by a system of anastomosing channels, which penetrate the cell-mass and open

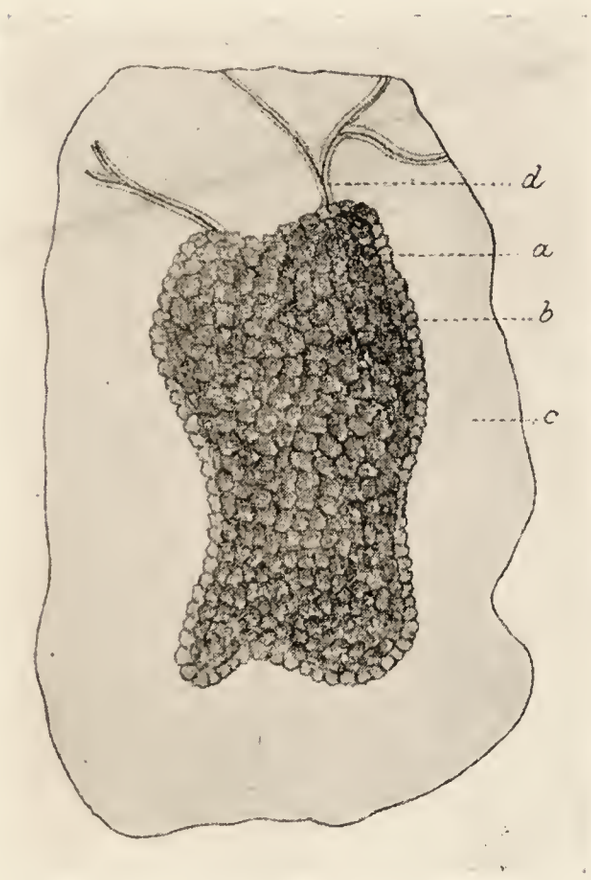

FIG. 11.—Red Gland of *Gadus morrhua.*

(*a*) Posterior part of red gland; (*b*) its yellow margin; (*c*) swim-bladder; (*d*) vessel. (Vincent and Barnes.)

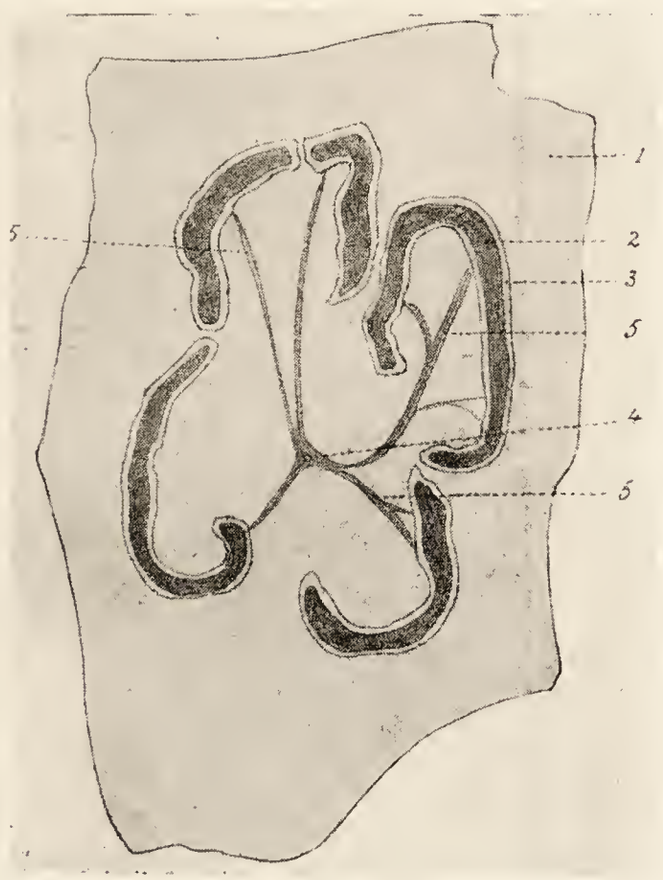

FIG. 12.—Red Glands of *Zeus Faber.*

(1) Wall of swim-bladder; (2) red gland, with (3) its yellowish border; (4) point of origin of vessels; (5) vessels. (Vincent and Barnes.)

into the bladder cavity by small apertures situated on the internal surface of the glandular mass (see Fig. 14).

The *rete mirabile* results from the intermingling of two sets of fine capillaries, those arising from the arterioles to the gland, and those from the venules. There is intimate contact between these but no intercommunication (Fig. 13). This rete mirabile may be *bipolar*, i.e. the arterial capillaries at the end of the rete next the gland may unite into a few large vessels before again subdividing to form the capillaries supplying the gas gland, the venous capillaries uniting in a corresponding

manner, or it may be *unipolar*, the arterial capillaries directly

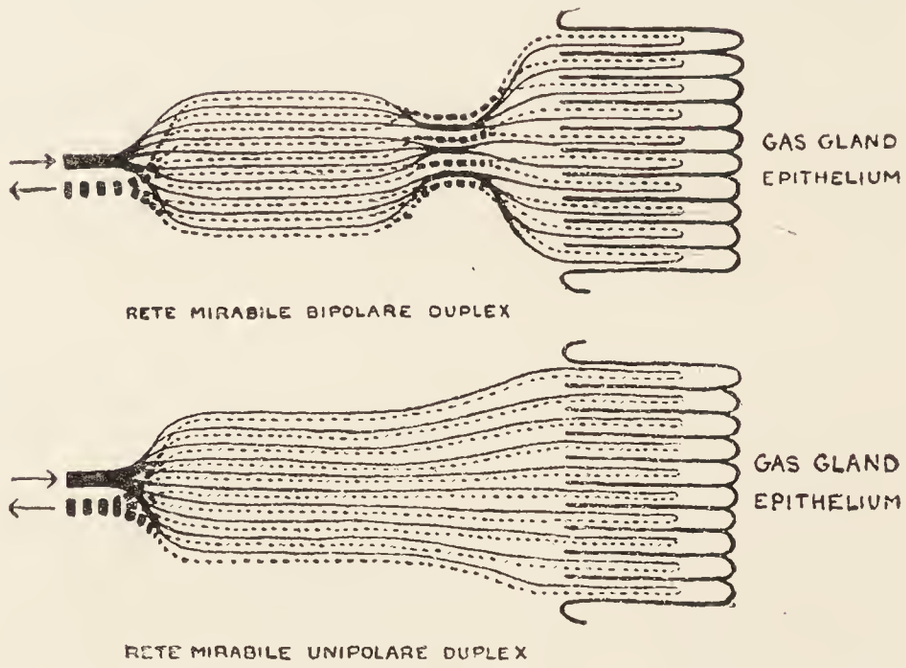

Fig. 13.—*Rete mirabile.* (Woodland.)

supplying the gland and the corresponding venous capillaries not so uniting into a few vessels (Fig. 13).

Fig. 14.—Glandular Epithelium of Gas-bladder in transverse section. (Woodland.)

Both the true glandular structure and the rete have been known for a very long time, but even at the present time it is

not possible to say with certainty *how* the double apparatus secretes oxygen into the bladder. The glandular cells present the general appearance of glandular cells elsewhere. There are frequently transverse markings and a granular aspect. The microscopical appearances are such as we are accustomed to associate with a liquid or semi-solid secretion. There is indeed no difficulty in detecting such a secretion. This can

Fig. 15.—Fish brought up from a considerable depth with Swollen Gas-Bladder projecting from Mouth. (Regnard.)

be collected and examined. It is viscid and slimy and of an opaque milk-white aspect. On microscopic examination it is seen to be granular and on chemical examination to contain a nucleo-protein. Whether this secretion has any relation to the gas secretion is not known (Vincent and Barnes).

Some observers have described gas bubbles in the gland cells and hæmolysis of the blood. But more recently the view has gained ground that just as in normal metabolism, the plasma

of the blood conveys all the oxygen derived from the oxy-hæmoglobin to the gas gland cells, and that these in some manner transmit it by a process of diffusion under great pressure into the bladder cavity (Woodland). Haldane [65] points out that in cold-blooded animals the dissociation of oxy-hæmoglobin in the tissues is practically dependent not on fall of oxygen pressure but on rise of CO_2 pressure. He suggests therefore that the function of the rete mirabile is to enable venous blood to communicate part of its CO_2 to the arterial blood. The effect of this will be to raise the CO_2 pressure of the blood supplied to the gland, and so raise the oxygen pressure. How the gland passes the oxygen into the bladder, or why, if the oxygen pressure were already raised, a complex gland should be required at all, are questions not yet answered. The secretion of oxygen in the gas bladder, like that of saliva, sweat, etc., is excited by the action of pilocarpine. The secretion which occurs after emptying the bladder by puncture ceases after the branch of the vagus supplying the bladder is cut (Bohr [24]).

Arcellæ and Difflugiæ are able to attain a higher level in the water in which they live by developing gas bubbles in their protoplasm. According to Bles (quoted by Haldane) want of oxygen is the stimulus to the formation of the bubbles. A slight fall in the normal oxygen pressure of the water in which Arcella lives is sufficient to cause the immediate formation of gas bubbles, and thus cause it to rise to where presumably there is more oxygen. The bubbles which are apt to develop when the animal is placed on its back are a consequence of stimuli produced by internal want of oxygen owing to increased oxygen consumption during its efforts to right itself.

Cameron [30] has observed that frogs immersed in water for long periods give off from time to time bubbles of gas from the mouth and the nares. This is practically pure nitrogen (with possibly a trace of CO_2). The gases are taken in through the skin and presumably liberated through the lung surfaces.

B. Hirudin

An interesting discovery was made by Haycraft [68] in 1884. It had been known for a long time that the bleeding which follows a leech bite is often difficult to check, and that the

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blood in the stomach of the animal does not coagulate, and that even after the blood is removed from the leech's body it does not clot. Haycraft found that an extract made with normal saline solution from the mouth region of the leech had the power of hindering blood from clotting, and this whether the material were injected into the blood-vessels of a living animal or added to blood *in vitro*.

The leech (*Hirudo medicinalis*) is not so commonly used in medical practice as was formerly the case. It may be recalled that in earlier days the physician was often called the "leech." The leech is common in ponds on the continent of Europe, and its normal diet is the blood of frogs and fishes. When opportunity arises, it fastens itself on to its prey and ingests enough blood to last for several months. The animal attaches itself by its hind sucker, brings the mouth into action, pressing the lips tightly on the skin and protruding its tooth plates. "Each of these tooth-plates is worked by muscles and is like a semicircular saw, for the edge bears from 60 to 100 small teeth. Rapidly these saws cut a triangular wound, whence the flowing blood is sucked into the muscular pharynx" (J. Arthur Thomson).

In passing down the pharynx the blood receives the secretion of the so-called "salivary glands." There are large granular pyriform cells surrounding the pharynx lying among the muscles of the seventh, eighth, and ninth segments. The cell is produced into a long duct opening out on one of the jaws. It is the secretion of these uni-cellular glands which prevents the clotting of the blood. This action is of advantage to the leech because there is no risk of blocking of the wound, and the blood in the storage pockets of the crop is rendered by its fluidity more accessible to the action of enzymes.

The active substance is not an enzyme. It is not destroyed by boiling or by alcohol. It has been stated that it is a deutero-albumose.* A preparation may be improvised by crushing leech-heads with sand, macerating in saline for two hours, and centrifugalizing. According to Sollmann "each kilogram of animal requires the heads of three leeches, extracted with 3 c.c. of normal saline. The solution loses activity on standing,

* This, however, is doubtful. Cf. the action of "peptone" injections in preventing clotting.

more slowly in the cold." Preparations of hirudin may be injected into a vein in doses sufficient to render the blood non-coagulable without injury to the animal, although death may result from the injection of its own defibrinated blood obtained by whipping.

Various methods of making dried preparations have been tried, but the commercial products are apparently at the present time not easy to obtain. It is said that many of them may become toxic from putrefaction if they are kept for some time.

C. **Silk and Similar Substances** (Spiders' Webs, etc.)

The silk-glands of the larva of the Lepidoptera are of great interest both commercially and physiologically. They are often referred to as silk-vessels or *sericteria*, and they differ greatly in their size in the various forms of the Order. In the silkworm each of the two vessels is nearly five times as long as the body. They grow very rapidly in the young larva, and undergo atrophy in the pupa stage.

The silkworm (*Bombyx mori* or *Sericaria mori*) is of Eastern origin but has now become domesticated in various parts of the world. Like all the Lepidoptera the larva of the silkworm possesses two long glandular tubes, which are derivatives of the ectoderm. Each gland consists of three parts: (1) a posterior convoluted part, (2) a middle part of greater diameter, and (3) an anterior part directed obliquely forwards, becoming narrow and joining its fellow of the opposite side forming a single terminal duct. On the course of the second portion, near its union with its fellow, are the two accessory glands of Filippi, of irregular form, the ducts of which may open into the final single tube. This last opens out on the ventral surface of the lower lip.

The middle and posterior parts consist of secreting tubes, while the anterior is simply conducting. The wall of the secreting portion is made up of flattish cells with extraordinary ramified and branching nuclei. These form very complicated patterns, especially in the cells of the middle portion. Their complication increases with the growth of the larva.*

* Such nuclei are common in the cells of insects.

These cells elaborate a complex viscous substance, which, under certain conditions, may accumulate in a visible form in the cytoplasm. The substance passes out through the cell wall into the cavity of the glandular tube and forms a cortical layer to the cylinder of viscous material which fills the organ. In this cortical layer a process of selection or separation occurs which is either chemical or physical, and which results in the isolation of a hyaline substance which accumulates in the centre of the gland tube in the form of a crystalline stem. This is drawn out by the silkworm into a fine thread which unites with that which comes from the other gland. In being drawn out these threads carry with them the cortical substance. The accessory glands probably either add something new to make the threads stick together or they serve the purpose of hardening the threads. The silk is not actually pushed out of the gland by the activity of the gland itself. It is removed by the silkworm, who having fixed the thread to some object, moves away and so pulls out the thread of silk.

During its emergence the thread passes through a small apparatus formed of a chitinous tube with an invaginated wall. This compresses the two threads together, and does so by the mere elasticity of its walls. But the compression may be varied or regulated by the action of muscles. The semi-fluid secretion is of a protein nature, and by dissection of the silkworms may be removed direct from the glands and so made into fibres of greater thickness than those made by the animals themselves. These threads are of great strength and are used for ligatures, fish lines, and other purposes (Helm [71], Gilson [59]).

The silk-glands of spiders are of various kinds and very numerous. In the common garden spider, *Epeira diadematа*, there are 600 glands, some aciniform, others tubuliform, while others are piriform. These open out on the "fusulæ" of the spinnerets. These are, to the number of half-a-dozen, placed beneath the abdomen, near its apex and immediately in front of the anal tubercle. They are usually arranged in three pairs, a posterior, a median, and an anterior. They are highly mobile jointed appendages, surmounted by the "fusulæ" or projections where the tubes from the glands open. In addition to the spinnerets and in front of them, some spiders

have an extra spinning organ in the form of a double sieve-like plate, the "cribellum." This is correlated with a comb of curved bristles on the metatarsi of the fourth pair of legs, the "calamistrum." The orifices on the "cribellum" lead to a large number of small glands, the "cribellum glands." The apparatus for spinning is thus very complicated.

The glands vary in shape and general architecture and many varieties are described. They consist essentially of a tunica propria and a secreting epithelium, but few histological details are forthcoming. A full account of the different kinds of glands in various species is given by Apstein [4].

In the act of spinning, the viscous fluid in the glands is squeezed out by compression, and there is a flow of liquid silk through the fine spools of the spinnerets. This solidifies as it comes in contact with the air. The thin filaments from each spinneret unite to form a thread, which may be joined to that from the others. It is said that the spider draws out the silk by its hind legs. It can regulate the rapidity of flow and can stop it at any time. The threads which are formed are exceedingly fine but quartz fibres can be made finer still. Space will not permit of an account of the mode of formation of the different webs. An excellent description of these will be found in the Cambridge Natural History [29].

Several attempts have been made from time to time to utilize the spider silk for commercial purposes, but it is said that the strength and the lustre of spider silk are inferior to those of silkworm silk. The real difficulty, however, seems to be the problem of providing suitable food for the spiders.

A very interesting form of secretion may be referred to here. The observations were made by Professors Cameron and O'Donoghue and communicated personally by Professor Cameron. The large sea-worm *Endistylia gigantea* found on the Pacific coast and reaching a length of eighteen inches, secretes its tube by means of glands along the whole length of the ventral surface of the body. If the animal be removed from its tube and placed in fresh sea-water, within twenty-four hours it pours out a thin viscid fluid along its whole length, and this slowly hardens (probably by contact with the sea water) into a gelatinous consistency, when it can be peeled off, exactly reproducing the pattern of the surface. The tube of the adult

worm built up in this fashion has the consistency of a $\frac{1}{8}$ in. white rubber tubing.

D. Poisons

Poisons are produced for the capture of food or for defensive or offensive purposes against enemies. Among poisons of animal origin may be named the various snake venoms, the poisons of amphibia, of fishes (e.g., the fugu poison of Japan), of scorpions, spiders, bees, ants, beetles (e.g. cantharidin) and other insects.

In ants the poison has been recognized for many years as formic acid. It has recently been discovered that only the *Camponotinæ*, ants without a sting and provided with a cushioned poison apparatus, contain and secrete formic acid. The males of all species possess no poison apparatus and do not secrete formic acid.

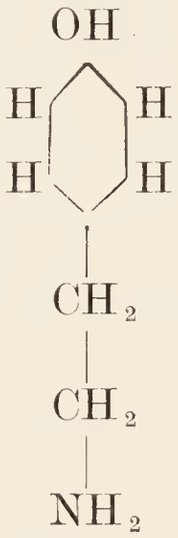
What is the nature of the poison injected by other biting and stinging insects is not known, though it may be imagined that mosquitoes could be collected in sufficient quantities in many parts of the world to make an investigation possible *

The mason wasp injects with its sting a substance into the nerve-ganglia of spiders, which paralyses the prey without killing it. The wasp deposits an egg close to the paralysed spider, so that, when the grub hatches, it finds fresh food, living but powerless, ready for its consumption (Warburton [122] quoted by Bayliss [12]).

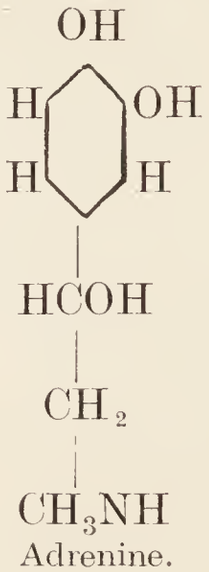
Henze [72] has investigated a poison employed by cephalopods. It has been known for a long time that a poison is produced in the posterior "salivary" glands of these animals, which enables them to paralyse crabs. The substance has been extracted and its pharmaco-dynamical action studied. Henze finds that it is para-hydroxy-phenylethylamine, and calls attention to the fact discovered by Barger and Dale [11] that this substance is produced from tyrosine by removal of CO_2 in the putrefaction of meat. He suggests the possibility that this may be the mode of production in the organism, CO_2 being split off during the activity of the gland. In support of

* An excellent account of the poison apparatus and the nature and action of the poisons in various insects is given by Marie Phisalix in *Animaux Venimeux et Venins*, Paris, 1922.

this view he quotes the observation of Schönlein [112] that an organic substance can be isolated from the acid salivary secretion of *Tritonium nodosum*. This, Henze says, is aspartic acid. Schönlein found on stimulation of the glands that CO_2 was produced. Bayliss calls attention to the chemical relationship between para-hydroxy-phenylethylamine and adrenine.



Para-hydroxy-phenylethylamine



Adrenine.

Snake poisons

Venomous snakes in the act of biting inject a poisonous fluid, or venom, the product of highly specialized, well-developed glands, which appear to correspond to the parotid glands of mammals. The fluid is injected by means of a series of specialized teeth having a groove or canal. These "venom fangs" communicate with the venom glands by means of the common gland ducts. When the snake bites, the glands are compressed by muscles and the venom passes along the common duct, through the groove or canal in the fang, and so into the tissues of the victim.

The poison glands are mostly "serous," but, surrounding the duct are a number of mucous glands, so that the parotid secretion of the ophidia differs from that of other vertebrates in that it has a considerable admixture of mucus. The epithelium is usually low cylindrical and the protoplasm granular, with some coarse granules. The nucleus is some distance from the base of the cell but never near the apex. In the epithelium immediately after a bite the nuclei are dark, stainable, not larger than half the diameter of the base of the cell and stand at some little distance from this. The granulation is gradually increased towards the upper part and thickest near the

free edge of the epithelium. In a snake which has been kept a long time and has not bitten, the granulation is much lighter and more evenly distributed throughout the cell-body. The process of secretion, as observed histologically, is thus roughly comparable to that of salivary secretion. According to some authors the nucleus takes an active part in the process.

Pharmaco-dynamically the snake poisons either act on the blood causing intravascular clotting and hæmolysis, or they cause paralysis of respiration by a curare-like action [96 38 54 55].

FIG. 16.—*Bufo agua.* Photograph of an Adult Male, showing the Variation in Tone of the Ventral and Dorsal Integument, and the Warty Appearance of the Animal.

The huge paratoid glands (*Pg.*) are shown behind the tympanum, and the pits into which the gland-ducts open are easily visible. (Shipley and Wislocki.)

Toad poison

Abel and Macht [1] in 1912 made some very interesting observations on the poison of the tropical toad *Bufo agua.* These authors have unearthed some very instructive items in the history of the subject. The toad has, from time immemorial, been regarded as a venomous animal. In the Talmud it is differentiated from the frog and classed with animals whose touch contaminates. For centuries the toad has been used

medicinally. The Chinese have long used as a remedy a preparation from toadskins which they call *senso*. This substance is said to have an action similar to that of digitalis, but fifty to one hundred times more powerful. This is especially interesting, as Western nations have used the dried toad as a remedy for dropsy. It is said that the skin secretion of toads has been used as an arrow poison, and it is certain that the poison of *Bufo agua* would be very deadly if used in this way.

In *Bufo agua* there are two large oval so-called "parotid glands" behind each ear. It is from these that the poison is obtained. From the secretion Abel and Macht isolated two distinct physiologically active crystalline principles. One of these is adrenine, and the venom contains 6·72 per cent. of this substance. The other is *bufagin*, $C_{18}H_{24}O_4$, which has a powerful digitalis action, increasing the tonicity of the heart and causing marked diuresis.

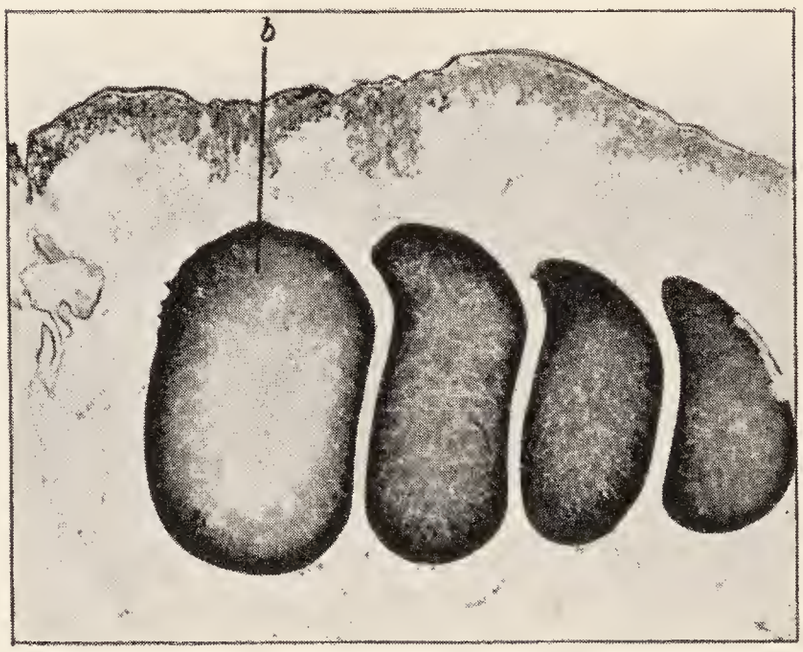


FIG. 17.—Section through the Paratoid Gland of an Adult Male Toad (*B. agua*). Photomicrograph. × 10.

The picture shows four mature gland acini lying embedded in the inner loose stratum of the corium and the depth of the cutis vera caused by their presence. These glands are full of secretion, ready for discharge. At the extreme left of the picture are the remains of an old discharged gland which is rapidly undergoing resorption. The thickening of the cutis is entirely due to a hypertrophy of the inner loose layer. *b*. The batteries of mature glands. (Shipley and Wislocki.)

The presence of adrenine as an "external" secretion is remarkable. It is not clear what effect the presence of adrenine would have on the poisonous action of the secretion.

The cutaneous glands of *Bufo agua* are of two kinds: (1) adrenine producers, and (2) those whose secretion does not contain this compound. The adrenine-producing acini are found only in the glands referred to above, the so-called "parotid" glands. When the contents of the poison-glands have been discharged, probably through the agency of smooth

muscle, the sac does not fill up again but disappears, its place being taken by an immature gland. The young glands bud out from the neck of the old sac. In maturation they grow downwards through the cutis vera, carrying with them a layer from the outer layer of the derma, and are surrounded by it during their existence.

The secretion of these poison-glands is produced by and during the destruction of the cytoplasm of epithelial elements lining the acinus of the young gland, which destruction leaves only a cell nucleus within the acinar wall. Two kinds of secretion are found in the lumen : (1) a granular secretion, formed first, filling the lumen in young glands and occupying the central part in the mature state ; (2) a clear, homogeneous or finely punctate fluid at the periphery of the mature acinus, which stains a bright yellow with chromates. This chromaphil reaction is not found in young glands, nor in the other cutaneous glands. Later on the chromaphil secretion extends into the central part of the lumen, so that the whole mass of secretion stains yellow with chromates. The clearer liquid is the first to reach the skin surface when the gland discharges, so that adrenine is the first poison to reach the mucous membranes of an attacking animal. The adrenine is not secreted as such by the epithelium, for the cells never show any chromaphil reaction, and the poison-sac does not contain any chromaphil material until long after the disappearance of all epithelial elements. So that the adrenine is probably the result of a change produced in a mother substance, possibly an amino-acid.

We have seen above (p. 65) that the poison of cephalopods is para-hydroxy-phenylethylamine and that it very probably results from the decarboxylization of tyrosine. It is suggested by Guggenheim and by Shipley and Wislocki that the mother substance of adrenine in these toad-glands is a compound of the same sort as tyrosine, and that adrenine is formed from it by a process of spitting off of CO_2. It is further suggested that the process takes place through the activity of the naked nuclei of the glandular epithelium.

The above account of the structure of the poison-glands of *Bufo agua* is taken from that of Shipley and Wislocki [113].

Brief mention must be made of the various powerful poisons

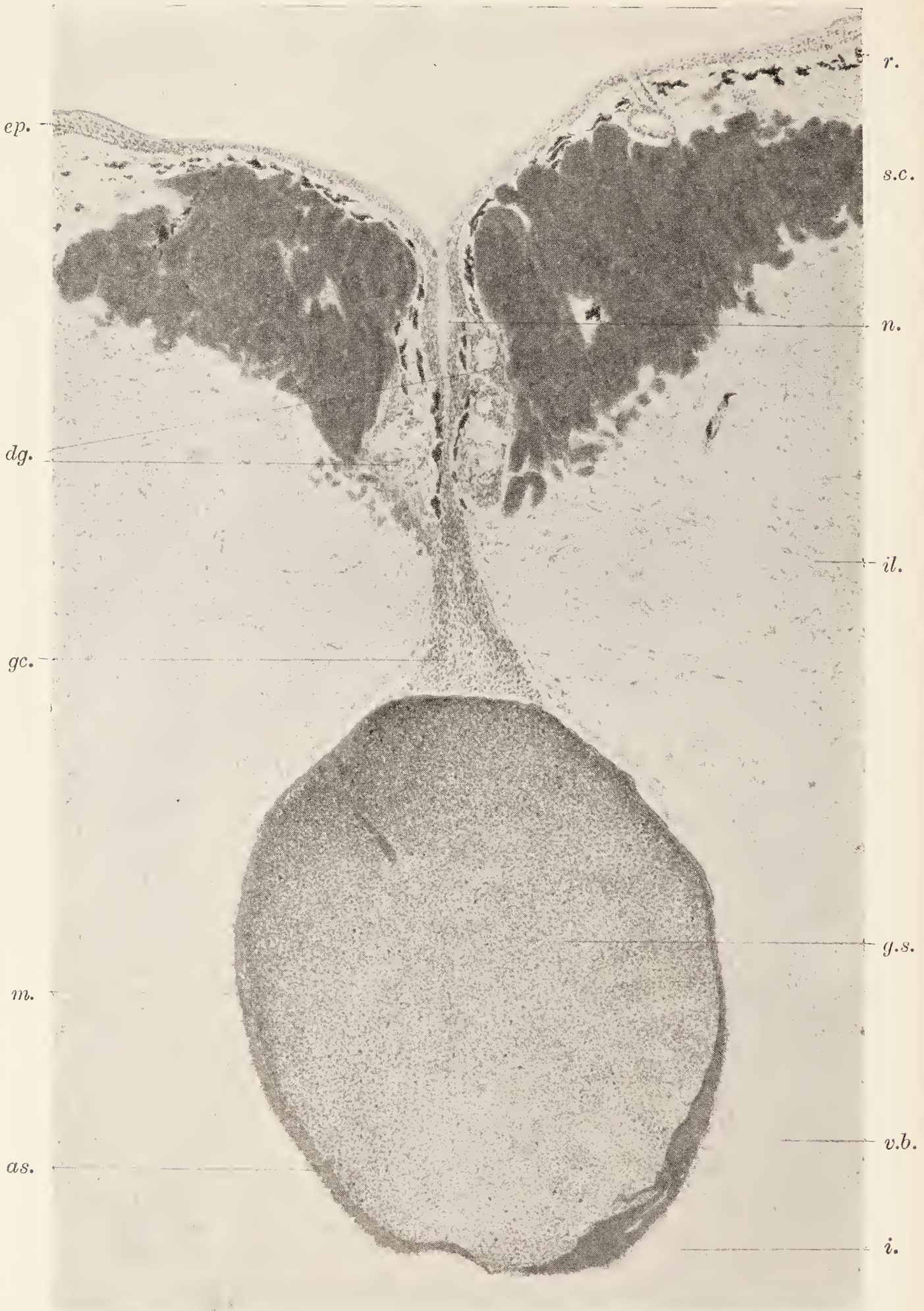


FIG. 18.—Section through the Acinus and Duct of a Mature Poison-Gland. Fixed in Formol-Bichromate (Mallory's stain). Freehand drawing, about × 100.

(alkaloids) found in the tissues of plants. Their mode of formation and the reason of their existence are alike unknown. They may be accidental products, or they may be of service in preventing their being devoured by animals. They are apparently purely intracellular "secretions," and only liberated from the cells by external injury.

E. The Ink-Gland of Sepia

The ink-sac of Cephalopods is a modified anal gland, and opens into the rectum close to the anus. Most of the bag is made up of connective tissue and some muscle fibres. But at the closed end, there is a distinct gland, constricted off. There are also glandular structures at the neck.

The inky fluid is poured out by the animal in order to enable it more easily to escape from its enemies. The well-known brown pigment belongs to the class of melanins. It may be obtained from various species of cuttlefish. The ink-sac is removed from the body and rapidly dried. The contents are dissolved in alkali and precipitated by acid. The precipitate after washing may be made up into various forms.

Description of Fig. 18.

The secretion in the lumen of this gland shows the yellow colour of a brilliantly positive chromaffin reaction. The homogeneous fluid about the periphery of the gland acinus gives the reaction most strikingly, but in this gland, which is evidently an old one, the epinephrin-containing fluid has diffused through the granular material in the centre of the acinus and the entire secretion contains some of the chromate-reducing material (epinephrin). This gland has about its duct a number of young glands growing downward into the inner loose layer of the cutis. The stratum compactum cutis appears as a dense blue mass. This particular section shows very well the dragging down of the corial melanophores by the gland as it carries with it, in its downward growth, a sheath from the outer loose layer of the cutis. (Shipley and Wislocki.)

Key to Figure References.

dg., developing glands.
n., gland duct.
gc., gland collar.
g.s., granular secretion.
as., layer of epinephrin-containing secretion.
m., muscular layer.
ep., epidermis.
s.c., stratum compactum cutis.
il., inner loose layer of the cutis.
i., zone of the inner loose layer of the cutis about the glandacinus.
r., melanophores.
v.b., vertical bundles of connective-tissue fibres.

von Furth is of opinion that the pigment is formed from tyrosine by the action of an oxidizing enzyme, tyrosinase.

F. Electrical Organs

It has been known for ages that certain fishes have the wonderful power of developing electric shocks. In the majority of cases the electric organ are transformations of skeletal muscles, but in the electrical cat-fish (*Malapterurus electricus*) the batteries are situated in the skin, the elements being unicellular glands.

The skin is thick and transparent, and invests the greater part of the trunk ; and is separated from the muscles by a loose connective tissue. The mass of the organ is made up of a gelatinous, yellowish substance, and accounts for one-third of the total body-weight. When examined under a lens the skin shows numerous small conical villi, between the bases of which there are rounded openings leading into tubular pits in the epithelium. Around these are disposed a number of bi-nuclear " club-cells, unicellular glands like those of other fishes : these empty their contents into the adjacent epidermoid tubes " (Biedermann [22]). The rest of the epidermis is made up of ordinary epithelial cells.

The electrical organ is made up of a number of small hollow spaces, which do not intercommunicate. These are lens-shaped and so placed that one wall is turned to the head-the other to the tail-end of the animal. On the posterior wall of each space is the *electrical plate.* This is homogeneous and nucleated, and supplied with a nerve-twig. The lateralis vagi of the common cat-fish is represented in Malapterurus by the *electrical nerve,* which is a single medullated fibre of giant dimensions which bifurcates and supplies the nerves to the compartments and electrical plates. It is supposed that the whole electrical organ has been derived from glands like those in the epidermis.

The organ can be excited to activity either directly or reflexly, and the peculiar arrangements result in a large multiplication of the electrical changes which, as we have seen, always accompany the process of secretion. The organ is

presumably used by the animal for killing or benumbing its prey or for purposes of defence.

In the *Torpedo* and the *Gymnotus* the electrical organ is developed from muscular tissue.

G. Luminous Substances

The production of light by living organisms is a fascinating though a very difficult subject. The literature is enormous and yet it must be admitted that we have not reached conclusions sufficiently definite to justify any systematic representation of the phenomena. A compendious account of the whole subject is given by Mangold [92].

Light is produced by many kinds of organism from bacteria to teleostean fishes, and even by some higher forms. The vast majority are marine. It is well known that certain putrefactive bacteria living on decaying fish or meat have the power of producing light. The same is true of certain fungi and a limited number of arthropods. In "phosphorescent" sea water, the light is due to millions of organisms belonging to nearly all classes of invertebrates. Among these *Noctiluca* has received most attention.

Many cœlenterates show phosphorescence, and among the crustacea many copepods have skin glands which secrete a luminous substance.

The boring mollusc *Pholas dactylus* has been long known to produce a luminous slime. The luminous organs are placed on the border of the mantle and elsewhere, and the cells have peculiar granular contents.

There are several instances of luminous insects. Some gnats and ants are luminous. The glow-worms and fire-flies are, however, best known. There is some little confusion in the popular terminology. Fire-flies is the popular name for the American click-beetles of the genus *Pyrophorus* (Fam. Elateridæ), of which there are nearly a hundred species. The Glow-worm is the wingless female of the beetle *Lampyris noctiluca*, common in many parts of England. The luminous organs in these coleoptera consist of groups of glandular cells in the ventral region of the posterior abdominal segments.

The light of the wingless female is said to attract the winged male, whose luminous organs are rudimentary or absent.

Light organs are present also in several other species of Lampyridæ. In the South European *Luciola italica* both sexes have wings and give out light. These are sometimes referred to as fire-flies. Again certain centipedes of the family Geophilidæ are luminous and are mistaken for the true glow-worms.

The light-organs of fishes have attracted a good deal of attention. Many deep-sea teleosts are phosphorescent. In some of them definite luminous organs are arranged in long rows along the body. The most elaborate have a cellular lens set in the opening of a cup which contains an epithelium of large cells. These are the cells which secrete the light-giving substance, and the walls of the cup, generally covered with pigment, act as a reflector, the whole arrangement having the character of a minute bull's-eye lantern.

Leuckart called these structures "accessory eyes." Leydig, to whom we owe the first accurate description, decided that they were not eyes. The histological structure is fairly uniform in the different varieties of fish, but there is great variation in shape, distribution, and origin. The essential part is a gland. Either the whole organ or a part of it is glandular. In the latter case the glandular portion is always proximally situated and other accessory structures of a special nature are added to it and form the distal part of the organ. So that the original form of the phosphorescent organs of fishes was that of a gland, which produced luminous slime. Many forms have a lumen and duct, while others have a lumen without duct. The cells show signs of secretory activity. In some forms the light is continuous, in others it is only produced in response to a stimulus. Sometimes special nerves can be traced to the organ, in other cases such cannot be found.

The phenomenon commonly called phosphorescence in living organisms does not require previous exposure to light, so it should be referred to a "*chemi-luminiscence.*" The light ceases in the absence of oxygen, so that it must be regarded as an oxidation. Water is necessary. In some cases, as in *Pholas*, the light can be produced by the slime

after it has been secreted and apart from the living animal, but in other cases, as in the organs of fishes, the light production occurs within the substance of the cells.

Langley and Very [84] investigated the production of light by the Cuban fire-fly (*Pyrophorus noctilucus*). Study of the spectrum of the light emitted showed, as might be expected from the colour of the light, that the maximum brilliancy is in the green near E. From this point the light falls away on both sides more rapidly than in the solar spectrum. The insect's light is unaccompanied by any measurable heat, and is thus a marvellously economic means of illumination. Nature produces this "cheapest light at about $\frac{1}{400}$ part of the cost of the energy which is expended in the candle flame, and at but an insignificant fraction of the cost of the electric light or the most economic light which has yet been devised." The certain small amount of heat which is developed in the active glands of the insect appears to be associated with the process of secretion.

Dubois [44] points out that the luminous organs are always ectodermia. In the medusæ they are, in fact, the epidermis itself. In myriopods, molluscs, crustaceans, and fishes they are glands with an internal or external secretion.* The production of light by the beetles Pholas and Cypridina is now known to be due to the interaction of two substances, luciferine and luciferase, in presence of water and oxygen. Luciferine and luciferase may be obtained separately by various methods. The luciferines and luciferases obtained from different species of animals have slightly different properties.

When luciferine from Cypridina (an ostracod crustacean) is oxidized there is no breaking up of the molecule, for oxyluciferine can be easily reduced back to luciferine. This substance has many of the properties of the proteoses and peptones, but its precise chemical nature is not known. Luciferase is a protein, and belongs to the group of albumins. Although it is used up in oxidizing large quantities of luciferine, it behaves in many ways like an enzyme and may be

* By this he means that the glands either have a duct, or have not. There is no suggestion that the secreted substance is absorbed into the circulation.

so regarded (Harvey [67]). A third substance, *photophelein*, is supposed to be capable of liberating luciferine from some bound condition in solutions containing luciferase. According to Harvey one part of luciferase in 1,700,000,000 parts of water will give light when luciferine is added, and a similar dilution of luciferine will give visible light when luciferase is added. It is possible that the reaction which results in luminescence takes place in two stages, similar to those supposed to occur in other enzyme actions.

$$\text{luciferine} + \text{luciferase} = \text{luciferine luciferase}$$
$$\text{luciferine luciferase} + 0(\text{or minus } H_2)$$
$$= \text{oxyluciferine} + \text{luciferase}$$

(see Harvey [67]).

In many cases of luminescence in animals the production of light has been shown to be due to symbiotic micro-organisms. These are found in the luminescent organs of certain fishes, cephalopods, tunicates and fishes. In 1922 Harvey discovered that the light organs of two species of fish (Photoblepharon and Anomalops) are made up of parallel gland tubes. The luminous material filling the lumen of the tubes is composed of an emulsion containing many granules and rods. The rods are bacteria and they move about with a corkscrew-like motion. It seems certain that the luminosity of the organ is due to these symbiotic bacteria.

Thus luminescence may be due either to the action of luciferase on luciferine or to symbiotic organisms.

H. The Secretion of Acid and Alkali

It is stated that *Dolium galea*, a mollusc, secretes sulphuric acid of the strength of 5 per cent. The acid is produced by a " salivary " gland and is supposed to be of use in breaking down the calcareous shells of its prey. Other molluscs use aspartic acid in the same way.

The presence of hydrochloric acid in the gastric juice has naturally aroused much discussion. Miss FitzGerald [51] has employed the double citrate of iron and ammonium with potassium ferrocyanide as a microchemical test for hydro-

chloric acid. When this is present in a strength of 0·1 per cent. the Prussian blue reaction is immediate and intense. The reagent was injected subcutaneously, and Prussian blue was found on the surface of the gastric mucous membrane, and in the voided urine. It was also found between the central lumina and the parietal cells and ramifying within these. This seems to be evidence of the actual presence of the free acid in the parietal cells, though the results have been called into question.

The cytoplasm of the parietal cells forms in itself the free acid, and under ordinary conditions the free acid so formed diffuses into the canaliculi and out into the lumen. But under certain conditions the free acid may diffuse in the opposite direction, i.e. out into the intertubular tissue. The source of the hydrochloric acid is the chlorides which are present in greater abundance in the parietal than in the chief cells or other adjacent tissue elements. The chlorine must be associated chiefly if not entirely with sodium.

It is difficult to imagine that hydrochloric acid is really produced within the living cell. Some attempts have been made to explain how the acid could be formed outside the cell. Koeppe [79] suggests that if the cell membrane is permeable to cat-ions ($H^{\cdot}$ and $Na^{\cdot}$) and impermeable to anions (Cl^{1}), acid might be formed outside the cell, and Bayliss [12] is of opinion that this is the usual state of the cell membrane as regards its permeability. But it is clear that much more information is required before we can offer any reasonable explanation of the phenomenon. Bayliss suggests that the surface action of colloids may ultimately afford an explanation when considered in connection with special arrangements of the cell membrane as regards permeability.

The secretion of alkali, as, for example, in the pancreatic juice, presents similar difficulties.

CHAPTER V

OTHER FORMS OF SECRETION

A. The Secretion of the Urine

An account of the functions of the kidney is included for the sake of completeness, and with the object of impressing the reader with the circumstance that the kidney is by no means a typical secreting gland. It excretes certain internally secreted products of other tissue cells. Its origin from the mesoderm and the extraordinary character of the histological elements, are alone sufficient to place it in a category by itself (see Fig. 19).

The difficulties connected with the physiology of the kidney are not of the same kind as those which face us in dealing with such an organ as the adrenal body. In the latter case we are still inquiring, " Of what service is the organ to the economy, what is the general function of the organ ? " About the kidney we can say quite definitely that its use is to get rid of certain waste products of metabolism. In all organisms, from the very lowest, there must be a mechanism for the excretion of substances like urea and uric acid which necessarily arise as breakdown products of protein metabolism. In the lowest unicellular animals such materials may be simply passed out through the cell-membrane into the surrounding medium. In some, a little higher in the scale, we find contractile vacuoles whose duty it is to expel waste material. In higher animals we have the nephidia. In vertebrates there are three elements to be considered in relation to the excretory system. In embryos at an early stage there are always traces of a *pronephros* (" head-kidney ") but it persists only in the lower types. The *mesonephros* forms the permanent excretory organ in forms below the Amniota. In higher animals the *metanephros* constitutes

the permanent kidney. In the human embryo there is a temporary pronephros at about the third week. Later the mesonephros is developed and gives rise to the reproductive organs. Later still arises the metanephros or permanent kidney. This arises from two distinct elements, a nephric or secretory, and a duct portion.

It is not necessary to describe in detail the structure of

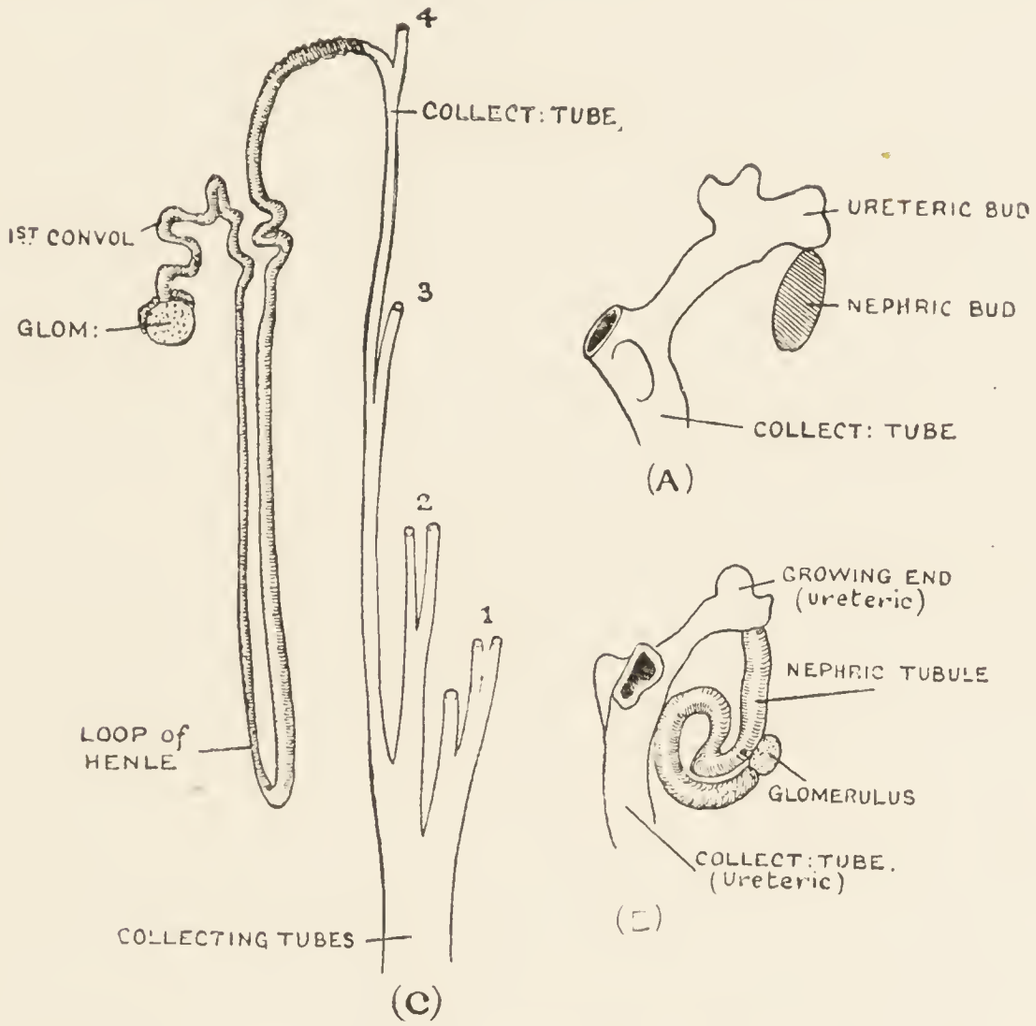


FIG. 19.—Development of Renal Tubule.

(A) Growing end of collecting tubule with nephric bud attached to it. (B) First stage of development of nephric bud into nephric tubule. (C) Fully developed renal tubule. The part formed from the ureteric bud is represented in outline; the part from nephric tubule is shaded (Keith).

the mammalian kidney. Most if not all the theories of kidney activity have been based upon the anatomical structure. The difference between the epithelium of the capsule and that of the rest of the tubule has led to such expressions as "the filtering part" and the "secreting part." The kidney is a collection of similar units, each consisting of a *capsule* with its *glomerulus* and the *tubule*. The epithelium of the tubule differs in different segments. Over the con-

voluted parts of the tubule and the descending limb of the loop of Henle the cells are tall columnar, "rodded" and ciliated. The lumen is small. In other parts the epithelium is of a thin pavement type, with projecting nuclei, or it is short columnar. The capsule is lined by flattened epithelial cells.

Since Heidenhain's account of the changes in the granules in secreting glands, numerous papers have appeared describing similar changes in the cells of the convoluted tubules during the stages of kidney activity. From *a priori* considerations it would not be expected that secretory changes of the character of those in a salivary gland would be found in the kidney. The kidney does not manufacture the substances it excretes, with the doubtful exception of hippuric acid, but merely extracts them from the blood. But it is not to be assumed that no histological changes in the cells would occur under these circumstances. The older observers described changes in the shape of the cells, in the distribution of the granules, in the "rods," and in the striated border. In some cases the cells were described as "loaded," in others as "discharged." Some have alleged that in a resting state the cells of the convoluted tubes have their granules arranged in rows and sharply marked off from the granule-free part of the cell, while during diuresis the granules become distributed irregularly throughout the cell. Metzner [97] describes the passage of drops through the striated border and their accumulation in the lumen. The drops stain feebly because they contain little coagulable matter, but here and there are darkly staining granules. These masses are connected with the cells by slender bridges.

These various appearances have been critically examined by Rathery [104] and Sauer [109], both of whom deny that they have any physiological significance. The act of secreting urine appears to have no influence on the structure of the protoplasm in the cells of the convoluted tubules. Heidenhain's "rods" (which are really rows of granules) and the striated border are to be seen in all phases of activity. The nucleus never changes its position. In fact the changes described by Disse, Metzner and others are artefacts, and the only observable changes which occur during secretion

are those which affect the lumen and height of the cells. When there is little flow the lumen is narrow and the cells high ; when there is abundant flow the lumen is wide and the cells low. Intermediate conditions may be observed. These changes most probably arise as a direct result of the distension of the tubes with water.

The blood-supply to a tubule is as follows :—

The arteries pass in a radial fashion to the cortex and give off side branches (afferent vessels) each of which breaks up into a small capillary nodule (glomerulus) enclosed in Bowman's capsule. The blood leaves the capillaries of the glomerulus by an " efferent " vessel, which is smaller than the afferent, and breaks up into capillaries round the convoluted tubules. This forms the only blood-supply to these tubules. This second set of capillaries ends in the efferent veins which unite to form the renal vein.

The mechanism of the secretion of urine is not yet understood. We have seen above (p. 29) that there are many reasons for believing that the liquid as it leaves Bowman's capsule and passes into the tubule is a filtrate from the blood, and in composition is that of the blood minus the colloids. During the passage of the fluid along the tubules, it is usually assumed that there is a selective absorption of water and certain substances.

Removal of the medullary portion of the kidney leads to the production of urine containing more water than in the normal flow. This points to the absorption of water by the tubules. If two salts, such as sodium sulphate and sodium chloride, are passed out together through the glomerulus, a process of re-sorption on the part of the tubules should affect chiefly the more diffusible salt, sodium chloride. Such a differential re-absorption would account for the much greater diuretic power of sodium sulphate as compared with sodium chloride (Meyer, Starling). Cushny induced diuresis by the injection of equal parts of equivalent NaCl and Na_2SO_4 solutions into the veins of a rabbit. Increased urinary flow was brought about and lasted two hours and a half. The chlorides in the urine increased with the increased flow, and reached their maximum at the same time as the latter. The chlorides then fell off and had largely disappeared from the

urine at the end of the experiment. The concentration of the sulphates, however, continued to rise in the urine up to the end of the observation (Cushny, Starling).

Such experiments show that the tubules have the power of modifying the fluid passed out by the glomerulus not only by absorption of water, but also that of dissolved salts.

Barcroft and Straub compared the consumption of oxygen by the kidney during normal secretion with that in diuresis

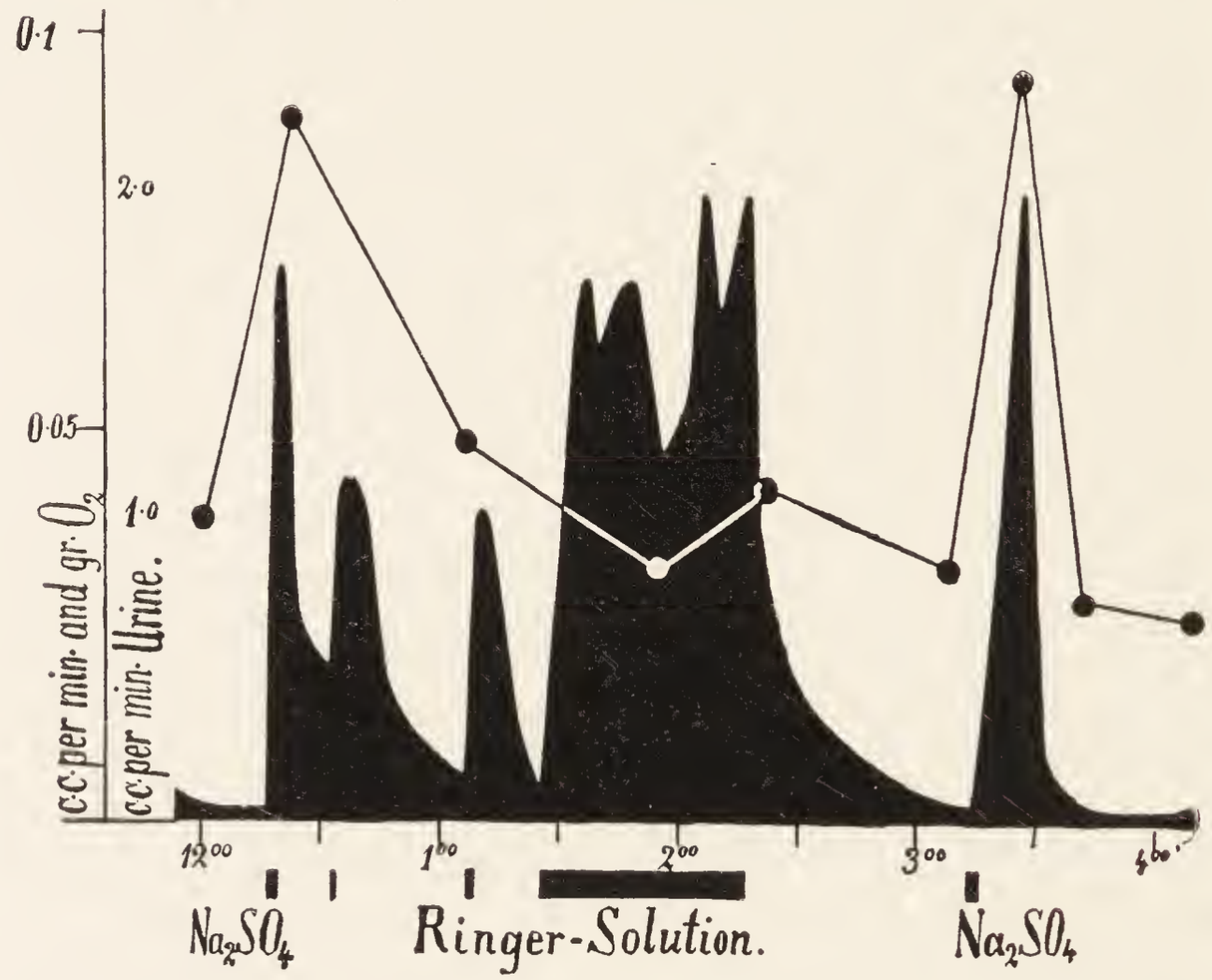


Fig. 20.—Line = Oxygen Consumption. Black area = Urine Excreted. (Barcroft and Straub.)

from the injection of Ringer's solution and hypotonic or hypertonic solutions of sodium chloride. They found practically no change in the oxygen consumption, although the amount of urine was increased nearly twenty-fold (see Fig. 20). A great increase in the fluid, chloride, and sulphate occurred with Ringer, yet the activity of the organ, measured by oxygen consumption, was unchanged. The observation undoubtedly tends to support the view that the increased flow of urine was due to filtration.

During the sulphate diuresis there was a marked rise in oxygen consumption. This Cushny thinks may be attributed to the work done in separating the sulphate from the water of the plasma.

The problem of filtration *versus* secretion in the capsule has been a vexed question since the time of Ludwig and Heidenhain, and although the great majority of physiologists have now given over their allegiance to the filtration theory, it would be rash to declare that the matter is settled. Among recent investigations supporting the filtration hypothesis may be mentioned those of Richards and Plant [107]. These observers perfused the rabbit's kidney *in situ* with whole birudinized rabbit's blood by a method which permits the pressure within the renal circulation to be altered without necessarily altering the total blood-flow through the organ. Changes in perfusion pressure induced by splanchnic stimulation, by introduction of adrenalin and nitroglycerin, by compression of the renal vein and by stimulation of the medulla, were accompanied by parallel changes in the rate of the urine elimination. The results constitute direct evidence in support of the filtration hypothesis of glomerular filtration.

Marshall and Crane [94] have recently contributed further evidence in the same direction. They find that the widespread belief that anæmia of the kidney of short duration produces a prolonged anuria is unfounded. Any arguments which have been used against glomerular filtration, if these have been based upon such a belief, are therefore of no value.

In addition to filtration in the capsule and re-absorption in the tubules, Marshall and Vickers [95] give good reasons for believing that there is a definite secretion in the kidney tubules. It will be recalled that from time to time arguments for secretion in the tubules have been put forward. A discussion of these is given by Cushny [37]. Marshall and Vickers believe that the elimination of phenolsulphonaphthalein by the kidney cannot be explained entirely on a filtration and reabsorption theory, but that the additional hypothesis of a process of secretion of the substance by the convoluted tubules is necessary. If phenolsulphonaphthalein be injected into a dog in which the elimination of urine had

been abolished by the low blood-pressure brought about by partial destruction of the cord, the kidneys are found to contain a much higher concentration of the drug than the blood or other tissues. As no urine flowed from the ureters it is probable that the substance is either concentrated in the renal cells or transferred through the renal epithelium to the lumen of the tubules while no urine is being poured out. Microscopic examination shows the drug is confined to the cells of the convoluted tubules. The authors conclude therefore that filtration cannot account for the high concentration of the substance in the kidney, but that it is taken up specifically from the blood by the cells of the convoluted tubules.

Marshall believes that all the non-colloid constituents of the blood plasma are filtered off by the glomerulus and capsule. Urea, phosphate, sulphate, and other bodies are added to this filtrate by processes of secretion on the part of the tubular epithelium. Water, chlorides, bicarbonates, and other substances are reabsorbed during their passage along the tubules. Certain substances which, according to Marshall, are formed in the kidney, ammonia (and perhaps creatinine) and other bodies are secreted by the tubules. The extent of the reabsorption of chloride depends largely on the concentration of chloride in the plasma, while the degree of reabsorption of bicarbonate depends on the hydrogen ion concentration of the blood.

Cushny thinks that it is not necessary to assume any process of secretion on the part of the tubules. This view, or one more nearly approaching that of Marshall given above, represents the working creed of most physiologists. But the literature is enormous and many writers hold views very different from those enunciated above. It is often objected that it is a clumsy and extravagant expenditure of energy to pour out seventy litres of fluid through the capsule and reabsorb sixty-eight. As an illustration of the diversity of views held by various workers, the work of Woodland [125] deserves attention. This author gives very good reasons for believing that the much talked of renal portal system in the frog has no function in the secretion of urine. It is merely a venous network derived from a purely extraneous structure which has become enclosed in the course of develop-

ment by the kidney tubules. The blood-supply to the tubules of the frog's kidney is essentially similar to that in mammals, that is to say, it is derived from the efferent glomerular vessels, the venous return from the tubules joining the venous network (renal portal system) which also happens to be carrying blood from the renal afferent veins, and which also happens to be imbedded in the kidney tissue. If this work be confirmed it would of course remove almost the only evidence of secretory activity on the part of the tubules, including the experiments of Nussbaum, and Bainbridge and Beddard.

As a result of very numerous experiments Woodland concludes that the glomeruli secrete neither water nor any other substance, the whole of the secretion being produced by the tubules. The encapsulated glomerulus is solely a pressure-reducing and current-retarding though volume-maintaining mechanism necessitated by the proximity of the kidneys to the aorta—the seat of maximum arterial pressure. But something must pass through the glomerulus if only through the difference of pressure. In this work of Woodland the morphological part will find more adherents than the physiological.

No attempt has been made to give a full and complete account of the functions of the kidney. From the papers referred to the reader will be able to get some idea of the present state of the problem.

B. The Secretion of Milk

It is usually stated that the mammary glands are modified sebaceous glands. In monotremes they appear to represent sweat glands, and some authorities believe that the same is true for all mammalian orders. It is suggested by some writers that they are not derived from either sweat or sebaceous glands but are organs *sui generis*. At first sight it would seem most probable that the mammæ are derived from sebaceous glands, but on chemical grounds it would be more tempting to suppose that they are of independent origin.

The glands, as is well known, are present in both sexes, but are normally functional in the female only. There is

great variation in number and position in different species. In the cow the mammæ are placed in the milk bag or udder, which has cisterns into which the ducts of the gland open. Each cistern opens outwardly by means of a teat. There are usually four of these, but there may be extra ones.

The mamma is made up of distinct lobes, separated by a variable amount of connective tissue and fat. The lobes are subdivided into lobules. Each lobule is made up of connective tissue binding together the convoluted ducts of the gland. Traced back these ducts are seen to be derived from groups of *alveoli*. Traced forwards they may be seen to unite to form the *lactiferous ducts*—fifteen to twenty in number in the human subject—which open on the exterior by minute apertures in the teat. These ducts at their origin from the lobular ducts become dilated, especially during lactation, to form *ampullæ* or *sinuses* which serve to collect the milk during the periods of glandular activity. The duct is made up of connective tissue with a small amount of unstriped muscle and lined by a columnar epithelium.

In the alveoli the epithelium differs in its appearance according to the state of activity of the gland. In the resting condition the lamina are wide and the cells form a single flat layer of granular cells containing a single nucleus. In the active state the epithelial cells become long and columnar, but irregular, and show appearances suggestive of division and budding off into the interior of the alveoli. There is an accumulation of granules and globules within the cells. These are partly of a protein and partly of a fatty nature. The alveoli become filled with a fluid containing detached cells and fatty globules. The detached cells are filled with granules staining with osmic acid and possibly identical with the colostrum corpuscles which occur in milk within the first few days after parturition. These latter, however, exhibit amœboid movements, and, according to Sharpey Schäfer [110], are rather analogous to salivary corpuscles and appear to be similarly derived from emigrated leucocytes.

The alveoli secrete milk during the period of lactation, not only during suckling but in the intervals also. In these intervals the milk accumulates in the alveoli and in the ampullæ of the ducts. It is stated that the udder of a cow

could not contain the milk which can be obtained at one milking, so that secretion of fresh milk must go on while the milk is being drawn. Further, the proportion of solids in the milk increases as the milking is continued. This may be due partly to the larger globules of fat meeting with greater resistance in passing through the ducts and so being held back till the end. Lehmann records that when a solution of sulphindigotate of soda was injected into a vein of a milch goat, no blue appeared in the milk until the udders were almost empty, when a slight blue tinge appeared. After an interval of an hour and a half the animal was again milked, when it was found that enough of the dye had penetrated to render the milk quite blue.

There has been much discussion as to the manner in which the secreted materials of the milk pass out of the secretory cells. The hypotheses which have been put forward are three, viz. :—

1. The cells themselves may break loose and become disintegrated, setting free their contents in the alveoli, as in the case of the sebaceous glands.

2. The cells may pour out their secreted materials into the alveoli as, for example, in the case of the salivary glands, without becoming detached or destroyed.

3. A part only of the cells, namely the free end, may break away and disintegrate. This third hypothesis supposes that the mamma in its mode of secretion occupies a position midway between the sebaceous and a gland like the submaxillary.

The first view undoubtedly arose from the belief that the mammary glands are modified sebaceous glands. According to this theory there is a complete fatty degeneration and disintegration of the whole of the secretory cells, their place being supplied by other cells which arise by the process of cell-division. The colostrum corpuscles are the detached epithelial cells. The chief objection to this hypothesis is that there is no evidence that extensive cell multiplication is taking place.

The second view is the one most generally accepted, and

enables us to regard the secretion of the milk as analogous in all respects with that of other secretions. The colostrum corpuscles are on this theory regarded as of the nature of wandering leucocytes (see above, p. 86).

The third view, that of partial disintegration, was first suggested by Langer, and adopted with modifications by Heidenhain and others. The cells become elongated so that their ends project into the lumen. The projecting parts disintegrate and the cell substance passes into solution to form the protein and carbohydrates of the milk. The fat droplets collecting in the disintegrating part of the cell give rise to the milk fat. The basal portion of the cell remains in position without being detached, and then develops fresh processes which later disintegrate. Some cells may simply discharge their fat droplets without disintegration. The more recent upholders of this view regard the colostrum corpuscles not as detached epithelium cells, but as wandering basophil leucocytes, which have wandered inward from the connective tissue (Schäfer [110], Marshall [93]). There seems to be no reason for excluding the hypothesis that in the initial stages of milk formation, cell disintegration occurs and then afterwards ceases when ordinary secretion takes its place.

Changes in the Golgi apparatus during lactation have been referred to on a previous page (p. 22).

There is no experimental evidence that the nervous system exerts any influence on the secretion of milk. Pilocarpine does not appear to have any effect, but atropine has a well-known power of drying up the secretion. It is stated that, short of stopping the secretion altogether, atropine, given in smaller doses, is found, whilst diminishing the amount of fluid secreted, to cause the secretion of a more concentrated milk (Schäfer). The composition of cow's milk depends on the breed, the stage in the period of lactation, the season of the year, frequency of milking, occurrence of sexual excitement, situation and climate, weather, and character of the food. The last is the most important factor. It is interesting that the administration of a diet rich in protein has a marked influence in increasing the fat of the milk. It has been sup-

posed that this is an example of animal fat manufactured from protein, but it may be that the protein stimulates the gland to increased general activity, causing the secretion of a large quantity of milk rich in all constituents—a " specific dynamic " action of proteins.

The chief organic constituents of milk are peculiar to the secretion, and do not occur in the blood or lymph. This shows that they are formed in the mammary glands themselves and not elsewhere in the body. It has been stated that a small amount of caseinogen may be found in the secretion of the sebaceous glands, from which, as we have seen above, it has been concluded the mammary glands have been evolved. The caseinogen may be produced by a change in the albumin or globulin supplied by the blood, or as has been suggested, from the degenerate nuclei of the gland-cells. But it is more probably to be regarded as a specific protein built up in the gland from amino-acids supplied by the blood. The milk fat is probably constructed from carbohydrate or may have its source in fat which has already been formed elsewhere and carried to the mammary glands in the blood or lymph. It has been thought that the lactose is derived from a glycoprotein precursor which, mixing with water and salts from the blood, contributes to the secretory product. But the mammary gland probably forms galactose from glucose and combines these to form lactose. Galactose is not formed as such elsewhere in the body, and when administered to an animal is normally converted into glucose by the intestinal mucosa and the liver.

The discharge of milk is due partly to direct mechanical pressure during sucking, and partly to the action of muscular tissue in the ducts and nipple. The muscular mechanism is called into action reflexly by the stimulus of sucking. But the flow of milk often occurs with a minimum of external stimulus.

C. The Secretions of the Skin

The skin glands of vertebrates are of varied kinds and widely differing functions. Some of them, such as the poison

glands of toads and the light organs of fishes, have already been referred to. They may be divided into two groups—

1. Those which secrete a watery solution, and
2. Those in which the gland cells break down to form the secretion.

In the first group are found the sweat glands of mammals and the slime secreting glands of fishes and amphibians. In the second we have to place the different varieties of sebaceous glands throughout the different orders of mammals and the uropygial gland of birds (see Fig. 21).

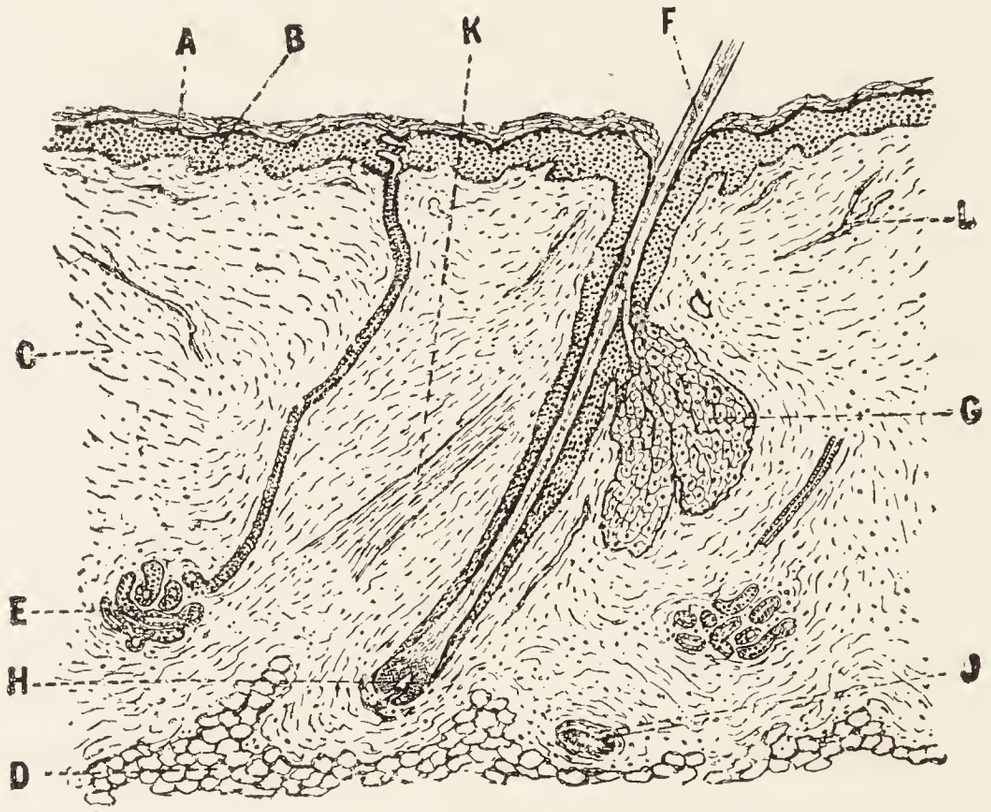


Fig. 21.—Section through the Skin (Low Power).

A, horny layer of cells; B, layers of soft growing cells; C, thick connective tissue coat; D, fat layer; E, sweat gland and duct; F, hair; G, sebaceous gland; H, papilla of hair; J, small artery; K, muscle of hair; L, capillaries. (Leonard Hill.)

The secretions of these glands are put to a great variety of use. The sweat serves to regulate the body temperature by evaporation and has in addition some excretory functions. The slime of the lower vertebrates is protective in function. The oily nature of the sebaceous secretion in mammals, or of the tail gland in birds, serves to protect the epidermal structures against the injurious effects of water. The secretion of the meibomian glands of the eyelids prevents overflow of tears. The viscous nature of the wax in the

auditory canal tends to prevent the entrance of foreign bodies. In certain cases odoriferous volatile substances in the secretions serve the purposes of sexual attraction or protection from enemies.

In the majority of hairy mammals sweating only occurs on special occasions and on certain parts of the body. Rabbits, rats, and mice are said not to sweat at all, the dog sweats but little, the cat only on the hairless pads of the feet. The horse, as is well-known, sweats abundantly on all parts, and the familiar panting of the dog is due to respiratory efforts to get rid of water, in the absence of perspiration through the skin. On the snouts of oxen and pigs are glands resembling sweat-glands, the secretion of which keeps the part moist (Waymouth Reid [105]).

Reid gives the following list of glands used for purposes of sexual attraction—the glands of the suborbital pit of many ruminants and some hogs, the cheek gland of the elephant, the pectoral glands of certain tropical bats, the flank glands of shrews, the sacral gland of the peccary, the groin glands of antelopes, the preputial glands of the beaver and musk-deer, the anal glands of the hare, marsupials, armadillos, two-toed sloth, otter, hyænas, and civets, and the glands at the base of the tail of shrews and the fox. Most if not all of these are of the general type of sebaceous glands. The anal glands of the skunk are used for protection.

Reid gives some further examples of skin glands. The gland which opens in the cleft between the two divisions of the hoof in ungulates is probably of use in protecting the horny matter from imbibition of water. In the one-horned rhinoceros a gland opens on the posterior aspect of each foot. In monotremes there is a gland on the back of the thigh in the male pouring out its secretion by a long duct to the hollow spur on the heel. It seems to be a poison gland but its function is not known for certain. In the male kangaroo *Halmaturus rufus*, there are skin glands which secrete a red material which sticks to the hairs, while the maxillary glands of the female dwarf antelope *Ophalolophus pygmæus* secretes a blue substance reddened by acid (Reid [105]).

There does not seem to be much recent work on these glands. Most of our information is derived from the descriptions of

Owen in 1868 and Leydig in 1850. Further work might necessitate modification of some of the above statements.

The water taken in by the food is excreted chiefly by the kidneys, but a small part leaves also by the lungs and by the skin. The amount got rid of by the skin is almost entirely dependent on the exigencies of heat regulation, and is not materially affected by the amount of water consumed along with the food. The water is partly evaporated from the surface of the skin, and when the temperature is sufficiently high partly by means of the sweat glands. These, as we have seen, are variously distributed in different animals. In man they occur over the whole surface of the skin, and are especially abundant on the palm of the hand and the sole of the foot. They consist of simple coiled tubes in the subcutaneous tissue, their ducts running upwards through the cutis to open on the surface by spiral channels penetrating the epidermis. The secreting part is made up of a basement membrane lined by a layer of cubical or columnar cells, which often contain a brown pigment. Between the epithelium and the basement membrane is a layer of fibres which are usually considered to be plain muscle. The secreting cells show striations and granules as in most glands. Minute canals have been described passing from the lumen of the tube between the opposed surfaces of the cells. Changes in the granules in different stages of activity have been described. In some of the larger glands two kinds of cell, one dark and granular, the other clear, have been stated to occur. In these larger glands may be found masses of yellow pigment.

The sweat is practically water. CO_2 is normally excreted in small amount, and is distinctly increased when sensible perspiration appears. Among the scanty solid contents sodium chloride is the most abundant, amounting to half the total ash. There is more ash in work sweat than in heat sweat, more in that from the chest than from the leg, and more from covered parts than from uncovered. The higher the temperature of the glands the greater the content in salts of the secretion (Talbert). Haldane and Moss (*Proc. Physiol. Soc.*, July 7, 1923) have recently given some interesting information on the physiology of sweating. They find that intense sweating at a temperature above normal causes almost

total disappearance of urinary secretion, and complete disappearance of chloride ions from the urine secreted. There is a correspondingly large secretion of chloride through the skin, and forty per cent. of the metallic ions excreted are potassium! The condition in extreme forms is associated with miners' cramp, firemen's cramp (in railway and ships' stokers).

The secretion is controlled by the central nervous system. The secretion may be called forth by reflex action or centrally. Central stimulation by warmed blood is the usual means of producing a flow of sweat. If one sciatic nerve in the cat be cut and the animal be put into a warm chamber, sweating will begin as the temperature rises in the intact paws, but not in the paw with the nerve cut. Sweating may be brought about in the cat's paw by warming the blood passing through the carotid arteries, though the blood passing to the paws may be of a normal temperature. If the sciatic nerve be stimulated there is a secretion of sweat, associated with constriction of blood-vessels.

Pilocarpine causes a production of sweat by a direct action on the gland cell and not indirectly by an action on nerve endings. But there is frequently a diminution of the response to pilocarpine of the glands of the cat's foot after section of the sciatic nerve. This change seems to be due to degeneration of the sensory fibres in the mixed nerve which in some way control the efficiency of the peripheral circulation. It has been suggested (Burn) that it may be due to an influence on the capillary tone due to adrenine in the blood.

The hypothesis of the existence in the posterior roots of inhibitory nerve fibres for the sweat glands has not been found to be tenable.

Adrenine causes no secretion of sweat, except when locally injected, and in this case the effect is due to the fluid and not the adrenine itself (Langley [82] [83], Burn [28]).

The sebaceous glands are saccular glands which pour out their secretion at the roots of the hairs. The secreting saccules are almost filled with epithelium cells, which are loaded with fatty particles. The deeper cells multiply constantly and, becoming filled with fatty granules, pass towards the centre of the alveolus. Here they undergo disintegration, and the

fatty and other substances with which they are charged form the secretion of the gland which is discharged by the duct into the mouth of the hair-follicle (Schäfer). The sebaceous glands are the only glands in which this process is now believed to occur.

Nothing is known of any nervous mechanism in relation to the secretion of the sebaceous glands.

The sebum consists of cholesterol esters with only traces of glycerides. The material is found in large quantities in sheep's wool, from which it is extracted and used commercially under the name of lanoline. This substance probably contains materials not derived from the sebaceous glands. It has the power of taking up water in large quantities, and it is not attacked by micro-organisms.

The function of the sebum is clearly to afford protection to the skin and hairs, from the drying effects of the air. Again its oily character will prevent the skin from becoming saturated with external moisture. It serves also to prevent undue loss of heat from evaporation of the sweat.

D. The Secretion of Tears

It is not necessary to describe the structure of the lachrymal glands. They are typical compound tubular glands provided with numerous ducts. The latter are lined with a double layered cylindrical epithelium, and lead to long tubules provided with low epithelial cells. These pass into the proper gland tubules, which are of the "serous" variety.

The secretion of tears clearly serves to keep the surfaces of the conjunctiva and cornea moist, and to help in the removal of foreign bodies. The fluid is a solution of sodium chloride and carbonate with some mucus and albumen. It is stated that the fluid has a certain bactericidal power, and that this is destroyed by boiling.

A secondary function of the lachrymal glands which has been developed in human beings is not easy to explain. Several glands are liable to have their activity modified in one direction or the other under the influence of emotion. This is very marked in the case of the tear gland. Under the influence of emotions (chiefly unpleasant) there is copious flow of tears.

The teleological significance of this is not clear. It is not known whether weeping is to be regarded as an emotional display or whether the process is in some way calculated to relieve the emotional strain. The latter is the popular view, but it must be remembered that the most powerful emotions do not give rise to a flow of tears. It is usually only when the violence of grief has somewhat subsided that the flow occurs.

CHAPTER VI

INTERNAL SECRETION

A. Introduction

In previous chapters we have been dealing with processes occurring in structures of very definite form and constitution which have long been known as glands, and which manufacture and pour out a product—the secretion, which we can weigh and measure and analyse. In the cells of these structures we can observe in many cases the stages of formation and elimination of the secretory products. Although, as we have seen, we cannot yet say what are the actual chemical and physical processes concerned, nevertheless we are able to attack the problems of secretion by methods which promise satisfactory results.

It is now our task to inquire how far what we have learned about secretion in general may be applied to what is generally called "Internal Secretion." It is justifiable to ask at the outset, whether the term secretion is strictly applicable in the latter case. It must have been borne in upon all workers on the subject that the processes termed "internal secretions" are of a fundamentally different nature from those of ordinary or external secretion. In the latter case the materials are passed out on to a free surface by means of a special set of tubes, the "ducts." In the former, the products of "secretion" are returned to the blood-stream. In many organs alleged to carry out such a secretion there is little to suggest secretory activity beyond a vague "glandular" appearance. It often happens that the glandular appearances are not equally obvious to different observers and a discussion arises as to whether a given mass of tissue is a gland or not. In such cases we have to rely largely on circumstantial evidence, as with the chromaphil tissues.

The profound differences between "glands" and "secretions" on the one hand and "ductless glands" and "internal secretions" on the other, has recently been pointed out by Sir William Bayliss in a review of a book by the present writer. He remarks:

"The name 'gland' implies to the physiologist many organs and tissues which have functions other than that of producing substances for the purpose of exerting a particular action on other organs or tissues when they pass into the blood-current. Those which form saliva and also the lymphatic glands may be mentioned. It is true that we might define a gland in a new way and say that any organ that produces some substance not already contained in the blood is entitled to the name. It would conduce to accuracy, however, if the name 'gland' were limited to those organs able to pour out a secretion which can be collected and examined —those of 'external secretion' in fact. In this case the ductless 'glands' would have to be called 'bodies' or some similar name, as is indeed frequently done in speaking of the 'pituitary body' or the 'suprarenal bodies.' The name 'secretion' itself as applied to the activity of the endocrine organs is also not very satisfactory."

With much of this there can be no dispute, but it must be urged that the terminological confusion has arisen from the circumstance that physiologists have used the term secretion to apply to several different processes. The chemical processes in the manufacture of products, the elaboration of these in suitable form for elimination, the passage of water and other materials out of the secretory cell into the duct of the gland and the final expulsion of the secretion on to a free surface —all these have been and are generally referred to as "secretion." If "secretion" be defined as including all these, then it is obvious that what is usually termed "internal secretion" has no right to the title. It follows also that the structures which carry out the process have no right to be called "glands," only "bodies." But Bayliss does not suggest what we are to call the process. The products he calls "hormones" or "chemical messengers." We shall refer to this again later.

The suggestion that "we might define a gland in a new

way " only reminds students of the subject of what has actually been done by many writers. The definition of a gland has been extended so as to apply to any structure made up of one or more cells of a special epithelial character which forms a product—the secretion—which is discharged upon a free epithelial surface, such as the skin or mucous membrane, or upon the closed epithelial surface of the blood cavities. But it has been pointed out that the substances manufactured by the tissue should be of a highly specialized character, and not ordinary products of metabolism if the process is to be called " secretion " and the organ concerned a " gland."

Perhaps one ought not to exclude from consideration the preparation by cells of specific chemical substances which will be used by the cells themselves in carrying out their specific function. As an example may be mentioned *glutathione* recently discovered by Hopkins [74]. This substance is a dipeptide containing the amino-acids cystein and glutamic acid. It brings about oxidation in muscle reacting with other substances in muscle (and present in extracts) forming a thermostable system capable of taking up ordinary molecular oxygen. This may be regarded as an " internal secretion " on the part of the muscle. The formation of such products, as indeed of intracellular enzymes, if it may be called " secretion " is much more " internal " than most of those usually so-called.

The subject of internal secretion has grown up within the last thirty or forty years. Before that time the text-books scarcely noticed the topic and the great majority of biological workers and medical practitioners and investigators had probably never heard the expression. At the present time the literature of the subject has become so enormous that a list of the titles of papers would fill several volumes.

There is scarcely a branch of biological science which does not now call in the aid of hypotheses derived from the realm of internal secretion. In medicine and therapeutics " endocrinology " constitutes the fashion of the day. The evolution of man is said to be modified by changes which have occurred in certain organs of internal secretion. The behaviour of human beings is attributed to variations in the functions of the ductless glands. Health, happiness and personal character

are all alleged to depend on the adequate and harmonious functioning of the endocrine organs. In books on the subject we find such headlines as "environment and heredity," "earliest impressions," "discipline," "nagging," "parents as teachers," and so on, and every conceivable topic concerning physical, social and moral welfare is dragged into the discussion.

B. History

The correlation of different organs and tissues of the body (the "consensus partium" of the older writers) was supposed by Cuvier, who died in 1832, to depend on the activity of the nervous system, and this we may suppose was the view current in his day. But in 1775 Théophile de Bordeu [25] had promulgated certain views which have been supposed to indicate a clear conception of internal secretion. This interpretation has been maintained by Neuburger [100] and by Biedl [23]. But Gley [62], who quotes in full the passages in question, is of opinion that Bordeu does not express himself so definitely as Neuburger believes. It is clear, however, that Bordeu looks upon each organ as the source of a "humeur particulière" which exerts its influence upon the body generally.

Gley [60] in 1897 called attention to the ideas formulated by Legallois [85], who, as long ago as 1801 entertained very definite views as to the relationships which exist between the different secretions and the variations in composition of the venous blood. The following is a translation of the text of Legallois :

"From the identity of the arterial blood and the diversity of the venous bloods, one may conclude . . . that the triumph of animal chemistry would be to find relations between the arterial blood, the matter of such secretion and the corresponding venous blood, both in health and pathological conditions in different animals ; to find the differences between the various venous bloods ; and, in short, to find these differences proportional to those of the corresponding secretions.

"Arrived at this degree of perfection, it would often be possible that the unknown was revealed by the equation : arterial blood = *such secretion + corresponding venous blood*

[the italics are the author's]—that is to say, the first number being given, it would be possible almost to guess at what ought to be the secretion if one knew the venous blood, or what ought to be the nature of the venous blood, if one knew the secretion."

As pointed out by Gley, Legallois' observations are general and apply to all glands and not only to those which ought properly to be called internally secreting glands. But this criticism is applicable to several recent authors [27].

Coming down to the work of Johannes Müller [99] in 1838 we find merely the somewhat cryptic statement that the glands without ducts can only exert a certain plastic influence on the fluids which circulate through them. "They consist, therefore, almost wholly of vessels."

W. B. Carpenter [32] in the year 1852 writes as follows :—

"There is another group of processes, which corresponds so completely with the secreting operations in its general nature, that it is difficult to avoid placing it under one category with them. . . . We refer to that elaborating agency, which is now generally believed to be exerted upon certain materials of the blood by the spleen, thymus, and thyroid glands, and suprarenal capsules (which are sometimes collectively termed vascular glands). . . . The 'vascular glands' . . . exactly correspond with ordinary glands in all that part of their structure by which they withdraw or eliminate certain matters from the blood; and they differ only in being unprovided with excretory ducts for the discharge of the products of their operation. These products, instead of being carried out of the body, are destined to be restored to the circulating current apparently in a state of more complete adaptiveness to the wants of the nutritive function."

It is, however, worthy of remark that Carpenter regards adipose tissue as having an internally secreting function. It will, I imagine, readily be conceded that the conception of internal secretion entertained by Carpenter is not vastly different from that which would have been accepted by the majority of physiologists a few years ago, and it corresponds fairly well with that maintained by many at the present time. It only differs from the most modern view in including the spleen and adipose tissue among the ductless glands. It

appears, then, that as far back as 1852 the main idea in the theory of internal secretion was present in the minds of physiologists, although the term "internal secretion" was not commonly used. The anatomists of this period appear to have been to a great extent responsible for the elementary conception of internal secretion, for, having decided that certain structures not equipped with ducts, were nevertheless in their essence "glands," they had no hesitation in postulating the flow of secreted material into the blood-current.

Caspar Friedrich Wolff (1733–1794) has been credited with the statement that "each single part of the body, in respect of its nutrition, stands to the whole body in the relation of an excreting organ" (Stewart [115]). A careful examination of the literature shows this quotation to be incorrect. There is a passage by Treviranus to the effect that "Each single part of the body, in respect of its nutrition, stands to the whole body in the relation of an excreted *substance*" (the italics are the author's) [119]. This sentence from Treviranus is, of course, of very different import from that attributed to Wolff (Paget [101]).

The first experimental demonstration of internal secretion was, without doubt, that of Berthold, of Göttingen, in 1849 [21]. This writer removed the testes from young cockerels and transplanted them on to the surface of the intestine. He found that young cockerels so treated do not develop in the way that castrated cocks do, but that they grow into normal cocks. The conclusion Berthold drew was that the "consensus partium" depends on the fact that the testes affect the blood and the blood affects the whole organism.* He was, however, of the opinion that the nervous system plays a part in the sequence of events. These observations of Berthold did not attract attention and they seem to have been entirely overlooked until they were unearthed by Biedl [23].

The doctrine of internal secretion as it is taught at the

* ". . . der fragliche Consensus durch das productive Verhältniss, der Hoden, d.h., durch die Einwirkung auf das Blut, und dann durch entsprechende Einwirkung des Blutes auf den allgemeinen Organismus überhaupt, wovon allerdings das Nervensystem einen sehr wesentlichen Theil ausmacht, bedingt wird."

present time was undoubtedly founded by Claude Bernard and Brown-Séquard. The term "internal secretion" was first used in 1855 by Claude Bernard, who referred to the glycogenic function of the liver as the "sécrétion interne," while he called the bile formation the "sécrétion externe." It is true that it is not now usual to treat the glycogenic function of the liver among the internal secretions. It is rather a special arrangement for the storing of carbohydrate food material. The glycogenic function of the liver is, however, intimately related to certain internal secretions—notably those of the pancreas and the adrenal body. Moreover, there are reasons for attributing to the liver certain other internally secreting activities. Bernard stated in the clearest terms that certain organs, such as the spleen, the thyroid body, the suprarenal capsules, and the lymphatic glands, furnish an internal secretion [19]. His work did not attract so much attention as that of Brown-Séquard on testicular extracts. This work, although of doubtful value in itself, aroused widespread interest in the problems of internal secretion. Brown-Séquard extended the meaning of the term far beyond the limits imposed by Bernard; he believed that all tissues furnish an internal secretion, and take part in the humoral function correlation of the body. This view is held by many at the present time, and will be discussed later.

Among recent discoveries which have advanced our knowledge of the subject of internal secretion may be mentioned the following: the association of myxœdema with disease of the thyroid, and the value of treatment by thyroid substance; the connection of diabetes with disease of the pancreas and the value of treatment by means of certain pancreatic extracts; the association of acromegaly and other disorders of growth and metabolism with disease of the pituitary body*; the pharmaco-dynamical effects of extracts made from the chromaphil tissues and from the pituitary body; and the isolation of adrenine and thyroxin. We must confess that the main conception of internal secretion has not been advanced and has only been slightly modified since the time of Carpenter (1852). On the other hand there has accumulated a mass of more or less disconnected facts and a tangle of unsupported

* See, however, Camus and Roussy [31] and Bailey and Bremer [8].

theories and unjustifiable practices especially in connection with what is called organo-therapy.

C. Nomenclature

We have already seen how the use of the term "internal secretion" has led to a disregard of the immense difference between secretion and "internal secretion," and in many ways the terminology of a subject has an important influence on the conceptions held by its teachers and investigators.

We have seen how the comparatively simple idea of "internal secretion" arose and became current after the time of Bernard. In 1905 Starling introduced the term "hormone" (*ὅρμαω*, to arouse or excite), to apply to the products of the organs of internal secretion. The word has been generally adopted and used instead of "internal secretion," which was previously used for both the process and the product. Bayliss confesses that the name is not a good one, and says that a short word with the meaning of "chemical messenger" is what is wanted. It is clear that philologically the word "hormone" implies neither a chemical nature nor a messenger's obligations. As pointed out by Schäfer, the expression "hormone" (*Ἑρμης* : Mercury) might have conveyed the idea of a messenger. The word "hormone" further, in meaning "setting into activity" has caused the introduction of a number of other names (*vide infra*).

But it is not certain that we need a word to mean "chemical messenger." At any rate it is by no means clear that such a term could adequately be applied to all or a large number of the internal secretions. The substances poured into the blood-stream by the organs of internal secretion do not deliver a message and then return to their central office. In many cases they stay where they are sent and perform the functions of workman, or agitator, or policeman or what not. In 1911 Gley [61] proposed the term *harmozone* (*ἁρμοζω*, to regulate) to apply to substances which serve to regulate chemical processes in the body. The harmozones are further divided by him into three groups: (1) Helping in nutritive exchanges; (2) serving to maintain the composition of the blood and lymph; and (3) having a morphogenetic function. According

to Gley, Starling's term "hormone" should be retained for specific functional excitants. Gley has also recommended for adoption another word—*parhormone*, to be used for such substances as CO_2, which he considers not to be true hormones, but waste products having an accessory excitatory rôle. Starling included CO_2 among his hormones. The blood serum is, of course, constantly saturated with CO_2 at the prevailing pressure. Increased CO_2 production in the tissues, or decreased rate of removal of CO_2 from the tissues (as in asphyxia) will lead to increased bicarbonate ions in the blood, but will even sooner produce a lowered pH (greater acidity) in all the tissues, including the respiratory centre. It is doubtful if there is any experimental evidence excluding such a simple pH change as the cause of increased respiratory movements. We can scarcely regard pH changes as "hormones."

When the word "hormone" began to be universally used, it was soon recognized that the substances called by this name do not all act as excitants. Some of them contain substances which have an inhibitory effect. This consideration led Schäfer in 1915 to suggest two new terms. The word "hormone," he points out, should be restricted to secretions which excite, while for those with inhibitory or relaxing actions he would use the term *chalone* (χαλαώ, to make slack or relax). For an expression to include both hormones and chalones Schäfer advises the term *autacoid substance* (ἄκος, a remedy, and αὐτος). So that we have *autacoid substances* divided into *hormones* or excitants and *chalones* or depressants.

These terms, while they appear to remove certain difficulties in the use of the word "hormone," have not improved the situation. In the first place the word "hormone" has been used in two different senses. The term "autacoid" has been criticized because the word drug indicates a foreign substance and not a material formed in the body. But a more serious objection is one which was pointed out by Schäfer himself, namely, that it is not feasible to classify internal secretions as exciting or depressing. Adrenine excites some tissues while it depresses others. Thus in small doses it causes the skin vessels to constrict while those of the underlying muscles dilate. Again the effect of adrenine on certain tissues may be either excitation or depression, according to the dosage.

Thus although moderate concentration of adrenine passed through the blood-vessels of skeletal muscles causes augmented venous outflow, larger quantities result in a diminished flow. A similar reversal of effect with change of dosage has been reported in the lungs (Hoskins [75]).

Many other expressions have been introduced during recent years. The word *endocrine* is now commonly used as an adjective applied to the internally secreting organs, their functions, and their products, and in antithesis to *exocrine*. The term "*endocrinous*" seems to be preferred by some, while *endocrinology* is the term applied to the subject of internal secretion. An *endocrinologist* is either a worker on the subject or a practitioner who undertakes to treat diseases arising from disordered internal secretions.* Transatlantic usage has made "endocrine" into a substantive, so that we frequently hear of the "*endocrines*" chiefly as applied to the (for the most part valueless) products of the manufacturing druggists.

Matthews has made use of the expression "*cryptorhetic*" (κρυπτος, hidden, and ῥεω to flow) as a descriptive designation of the internal secretions. This term has the very great advantage of being non-committal in respect of any theory as to the way in which the substances act. It is in this respect better than *endocrine* (κρινω, to separate) or "internal secretion," for both these terms imply some kind of "secretion." "*Incretion*" is now frequently used. If its antithesis *excretion* be used, it would mean ordinary secretion. Noël Paton [102] considers that many of the terms in general use are unsuitable, and that the substances contained in the internal secretions play a part similar to that played by the salts of the blood in maintaining the efficiency of the heart's action. "A certain minimum amount of each seems to be essential, and some proportion between the amounts of each must be maintained if the metabolism is to continue in its normal course. . . . Such a conception is more in accordance with the facts which we possess than that of a series of hormones or excitors calling forth the activity of the various tissues." This conception agrees well with what we know of the part played by the

* These latter terms are commoner in America than in Europe, though there are some people in England who style themselves "clinical endocrinologists."

active principle of the thyroid body in relation to the metabolism of the organism. Kendall regards the thyroid principle as a catalytic agent. As recently pointed out by Starling [114], any general statement that the actions of the internal secretions are catalytic would be unjustified, although it is possible that certain reactions brought about by their agency may be found to depend on catalysis. Since we do not yet know how these bodies produce their effects on the different functions of the body, we may fervently hope that investigators will for a time abstain from coining new words.

CHAPTER VII

THE EVIDENCES FOR "INTERNAL SECRETION"

A. Introduction

From the historical account given above a general idea of the findings which have given rise to the conception of internal secretion may be formed. We have already called attention to the essential differences between ordinary or external secretion and the process which has come to be called "internal secretion." It is becoming more than ever necessary to examine with great care the evidence which justifies us in asserting that any particular organ or tissue manufactures and pours into the blood current an internal secretion. This necessity arises from the circumstance that the function of internal secretion is often postulated on quite insufficient evidence. Sometimes what is supposed to be a "glandular" appearance of the tissue is considered sufficient evidence of the process of secretion. More frequently the presence of any substance in the gland itself which can be recognized chemically or pharmacologically is regarded as proof that such substance is poured out as a secretion. This last is the common fallacy in the method of injection of organ and tissue extracts. It is clear that three conditions must be fulfilled before we can place any given structure definitely and certainly among the organs of internal secretion. There must be histological evidence of secretion in the essential cells of the tissue in question. Some special chemical substance must be recognizable in the cells and in the efferent blood or lymph. This substance must be proved to show some influence not common to preparations of organs and tissues in general. In some instances, as in the case of the thyroid and pancreas, indirect evidence was so powerful that it was generally

agreed that a process of internal secretion occurred, though the above-mentioned criteria were not forthcoming. At the present time we have to face problems connected with the adrenal body (cortex), the chromaphil tissues, and other structures where the experimental data have not led to a definite conclusion. In fact, the evidence of this kind might reasonably in some cases make one hesitate to admit that we have to deal with internal secretion. In such cases the histological evidence may eventually prove to be of great value.

If it is found that when a certain organ is extirpated, the operated animal always shows a definite train of symptoms, and if when the gland is replaced in some part of the body other than its original position, or when extracts of it are administered to the animal, the symptoms disappear, we have strong circumstantial evidence of what is usually called internal secretion. The inference that in such cases the organ keeps the animal normal by means of a chemical influence is almost unavoidable. Such a chemical influence is at the present time generally called "internal secretion." The thyroid is the best illustration. In the case of the adrenal body the state of our knowledge is very different. Extirpation of the cortex is fatal, but we know nothing of any special substance manufactured by it. Extirpation of the chromaphil tissue contained in the adrenal is not fatal, possibly because chromaphil tissue is present in other parts of the body. On the other hand the tissue contains adrenine, a substance of well-known chemical composition and possessing marked pharmaco-dynamical properties. It is certain that the adrenal medulla pours out a certain quantity of adrenine into the blood-stream, that this quantity varies from time to time, and is under nervous influence. The animal can live quite well under normal conditions without the passage of adrenine into the blood, but it may be that its presence is called for in certain physiological emergencies. These two organs have been briefly discussed in this place in order to give some idea of the general nature of the problems which confront us.

CLAUDE BERNARD.

B. The Histological Evidences of "Internal Secretion"

1. *The Thyroid Body*

The thyroid is built up of a number of closed vesicles containing a viscid substance, the "colloid." The vesicles vary very greatly both in size and shape. They may be exceedingly small and almost devoid of lumen, while in other cases they may reach a diameter of hundreds of microns. The shape of the vesicles or follicles depends to a great extent on the pressure to which they are subjected. They may be spherical, and this is the commonest form, or they may be tubular or saccular. They are held together by loose connective tissue, and the whole gland is contained in a firm capsule.

In many lobules one finds in addition to the vesicles solid cords and nests of epithelium cells, and these are more numerous in the young and developing thyroid. This intervesicular cellular material varies within very wide limits in the thyroid of different species of animals, in different individuals of the same species and, to some extent also, in different regions of the same gland. It is not rare to find a pair of vesicles in close juxtaposition to each other, so that the colloid of one is separated from the colloid of the other by nothing more than two rows of vesicular cells. In other cases there is a certain amount of connective tissue separating the vesicles; but it is more usual to find, separating the colloid vesicles from one another, a variable mass composed of cells which are almost identical in size, nature of cytoplasm, size and form of nucleus, with those lining the colloid vesicles. It has been stated that they become rearranged into secreting follicles in cases of exophthalmic goitre. It would be natural then to regard them as embryonic thyroid cells which may develop into secretory elements as the demand arises. But it has not so far been possible to prove that such is the case. It is said that they may originate adenomata. They closely resemble the cells of the parathyroid glands. But it must be remembered that the latter structures develop from a quite different Arlage. There are also cells of other kinds between the colloid vesicles. There are lymphocytes and

isolated portions of thymus IV, in connection with parathyroid IV. There are also cystic cavities with ciliated epithelium, the remains of the post-branchial body.

The colloid substance, stored up in the follicles, is seen in fresh preparations as a clear viscid substance. Very numerous investigations have been made upon the microchemical reactions of this colloid material. For an account of these see Vincent [121]. In this place it is only necessary to state that when the material is properly fixed the colloid stains deeply with eosin and entirely fills the cavity of the vesicle. It is said that a small fraction is basophile. There are two appearances in sections of the thyroid familiar to all students of the organ, which require a word of explanation. The cells frequently show a vacuolated appearance due in all probability to drops of a fluid of different character from the rest of the colloid. These are stated to be derived from solution of desquamated epithelial cells. There is often a regular arrangement of one for each cell, suggesting a secretion on the part of each cell of a material which is more fluid than the rest of the colloid. The other appearance referred to is an irregular crenated border to the colloid. This is usually attributed to shrinkage from faulty fixation. Freshly secreted colloid is more fluid in consistence and does not stain so deeply as that which has been formed for some time.

According to Langendorff the epithelium cells are of two kinds—"Hauptzellen" and "Colloidzellen." In osmic acid preparations stained with the Ehrlich-Biondi mixture, the former are unstained, while the latter appear red with green nuclei. Other observers have not been able to satisfy themselves that these represent two separate kinds of cell such as we find in the stomach. Cowdry [33] says that it is generally admitted that the colloid cells of Langendorff, which are sometimes present, are undergoing degeneration, being in the last stages of cytomorphosis. Bensley has described two distinct cell types in the opossum, but these do not seem to correspond to the two kinds of Langendorff. If it should be found that there are really two types of secretory cells, the possibility of a dual secretion would have to be considered.

The cytology of the secreting cells and changes during activity.—By ordinary modes of preparation the cell protoplasm shows a reticular structure with frequently a longitudinal striation. The nuclei are spherical and show distinct nucleoli. In the cytoplasm are to be seen droplets of colloid and sometimes fat droplets. According to Cowdry [33] the mitochondria are hard to distinguish in a fresh state, but may be readily revealed by Janus green. They are arranged in the form of filaments parallel to the long axis of the cell and are most abundant in the distal zone of the cell, next to the lumen. In discussing the usual criteria of over activity on the part of the thyroid (increased height and size of cells, absence of colloid, etc.), Goetsch suggests that the mitochondria may prove to be a better index of thyroid activity than the histological criteria heretofore applied. Since the mitochondria occur normally in the thyroid cell and since they are present in greatly increased numbers in the adenomata associated with symptoms of hyperthyroidism, it would seem probable that they are correlated with an overproduction of an otherwise normal secretion produced by the thyroid cell.

The Golgi reticular apparatus (see p. 21) may be seen, after appropriate methods of preparation, as a circumscribed network placed between the nucleus and the lumen. But it is not yet certain that a similar network occurs in the living condition.

Quite recently Williamson and Pearse [123] have described a network of tubules stretched beneath the free surface of the epithelium. Occasional radial branches from the system pass towards the base of the epithelium. When secretory activity is obvious in the parenchyma these tubules are distended and contain granular material. The structure is described in the thyroid, but occurs in other secreting epithelia (see Fig. 22).

These authors have recently published some new views on the general structure of the thyroid. They believe that the lobule of the gland is made up of a collection of units, each one of which represents an expansion of the lymphatics of the organ, and forms a *lymph sinusoid.* Within the limiting endothelial membrane of the sinusoid lie coiled and convoluted columns of

thyroid epithelium, enmeshed in their specific plexus of blood capillaries; both epithelium and capillary endothelium are entirely unsupported by connective tissue. The interfollicular tissue is essentially thyroid epithelium. Two distinct cycles of function are postulated, one in the passive process of *vesiculation* whereby the reserve of colloid is stored within the epithelial column, the other that of active secretion in which a substance different in nature from the colloid is elaborated by the epithelium. During the process of vesiculation colloid accumulates under tension at discrete situations in the column: the distension of these discrete areas finally distorts and fragments the column, giving it the appearance of a series of vesicles. Williamson and Pearse consider that it is the predominance of this cycle of function in the average normal gland which has obscured the essential morphology of the organ.

According to these authors the secretory process is one of intense activity in the epithelium—an activity closely comparable to that seen in other secreting epithelia, e.g. mucous epithelium. In the thyroid it culminates in the production of a lake of colloid-like fluid within the epithelial column; hence the term "lacunation" has been used to describe the culmination of the secretory phase in contradistinction to "vesiculation" of colloid. The gland unit as a whole participates in the secretory phase; fluid appears in the sinusoid separating the epithelial surfaces; the endothelium of the lining wall and of the contained capillaries becomes active; lymphocytes appear at the hilus, and can be seen passing away along the lymphatic channels. No such phenomena are seen in the phase of colloid storage. A consideration of the two cycles leads the authors to regard the colloid as a vehicle in continuous circulation (analogous to certain constituents of the bile), the excess or reserve of which is stored within the resting epithelium. The vehicle carries a metabolite to the sinusoid. Here the epithelium deals with it, and passes the colloid either back into the circulation or into store. (*Trans. Path. Soc.*, July 1923.)

Bensley [17] thinks that he has observed by a special method (formaline—Zenker, brazilin and water blue) the true antecedents of the thyroid secretion. These occur in the form

of vacuoles and only in the proximal part of the cell near the blood-vessels. These are said to contain a dilute solution of a nature resembling that of the colloid. Bensley believes that the polarity of a gland cell has been reversed in the case of the thyroid ; so that it now secretes in the direction of the blood-vessels round the vesicle and not always into the vesicle itself. He makes a comparison with the condition of things in the pancreas which has a proximo-distal polarity —substances passing from the blood through the basement membrane into the cell and through the cell distally into

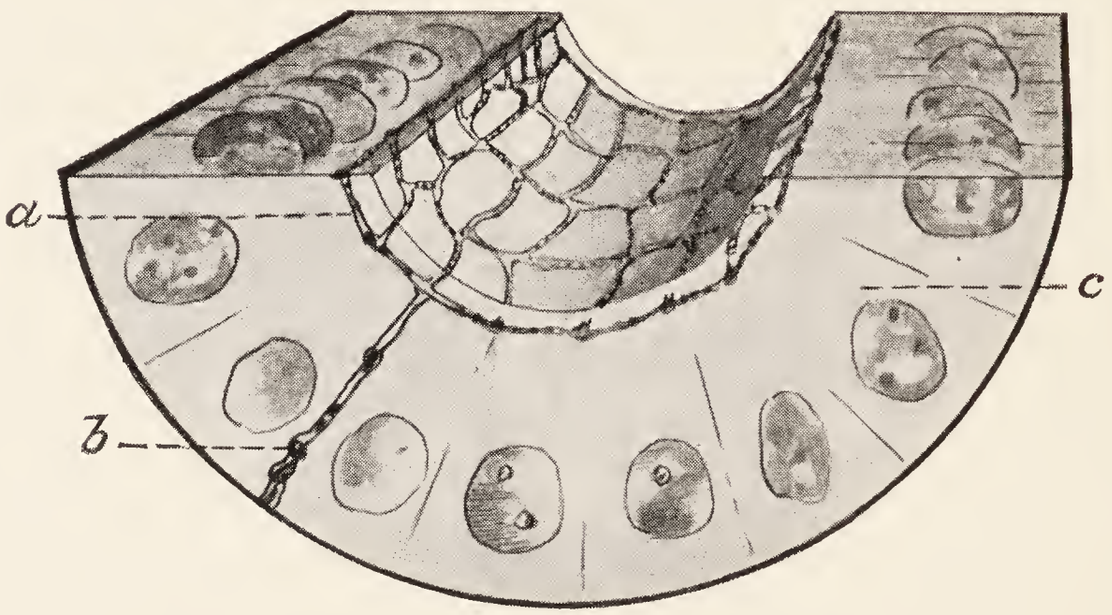


FIG. 22.—Schematic drawing of a Secreting Epithelium, showing the Position of the Tubular Network.

(*a*) Tubular network ; (*b*) very occasional radial branch ; (*c*) cytomitome. (From Williamson and Pearse,)

the duct. In the thyroid the condition is reversed, materials passing from the blood-stream into the cell, then out of the cell again and back into the blood-stream. He points out that the forerunners of the secretion are placed next to the basement-membrane in the thyroid, while in the pancreas they are heaped up near the lumen. When the secretion is in excess of the demand, it is passed in the reverse direction into the follicular cavity. According to this conception of thyroid secretion the colloid in the thyroid vesicles is *per se* no measure of the activity of the gland at the moment of observation, though its consistence and its qualities may

offer some indications of the capacity of the thyroid for normal storage. The secretion is normally poured directly into the blood-vessels and lymphatics.

Cowdry [33] finds that the Golgi apparatus moves actively from one pole of the thyroid cell to the other under normal conditions. He thinks that this is a visible expression of secretory changes in the cell. These changes are more frequent when the epithelium is high and columnar. Similar changes in the Golgi apparatus occur in the gastric and salivary glands and, as we have seen, in the mammary gland.

As to whether the secretion of the thyroid is continuous or intermittent, there is not sufficient anatomical evidence to enable us to decide. There seems to be no regular periodicity in these "ductless glands," as there is in others.

Certain reactions to experimental conditions have been described. Various observers have reported changes in the thyroid with variations in the diet, as depending on the activity of the other "ductless glands," during inanition, changes of temperature, unhygienic conditions, and under the influence of drugs. In considering such changes it must be admitted that the appearances are very confusing and difficult to interpret. The normal variations are much greater than is usually suspected. The mitochondria and the Golgi apparatus should be carefully studied in all future work of this kind.

2. *The Parathyroid Body.*

The cells forming the parathyroids are arranged in columns or clumps separated by bands of connective tissue. These columns and clumps vary in their grouping even in different parts of the same gland. Sometimes the connective tissue is abundant, sometimes sparse. Occasionally there are well-defined follicles containing colloid. Whether this is of the same nature as the thyroid colloid has not been determined. But the appearance of a parathyroid containing colloid vesicles is strikingly like that of thyroid, though the vesicles are smaller. It is not known what are the conditions which give rise to the formation of these colloid vesicles in the

parathyroid, but I have seen one case in the human subject and have published a drawing showing the vesicular structure (Vincent [121]).

Two types of cell are described in the parathyroid: (1) principal cells; (2) oxyphile cells. The principal cells make up most of the tissue and have a clear cytoplasm and faintly staining nuclei. The oxyphile cells stain deeply with eosin and their nuclei stain deeply with nuclear stains, as, for example, hæmatoxylin. These are thought to be cells in a state of degeneration as they do not occur in the parathyroids of young animals. Glycogen may be found by microchemical tests in both kinds of cell, but more abundantly in the principal cells. Fat is present in variable amount and increases with the age of the animal.

It has been stated that the mitochondria in the principal cells become converted into the material of the secretion, but the evidence on this point is by no means convincing. The hypothesis has been put forward that the actual secretion is of a lipoid nature and arises by a swelling up of the mitochondria.

The Golgi apparatus shows great variations in position in different parathyroids, but it does not seem to be definitely polarized for secretion in any one direction, though occasionally (when the cells are arranged in clumps) there is a sort of polarity and the apparatus takes up a position between the nucleus and the discharging pole of the cell. The ground substance of the cell does not show any signs of the presence of the antecedents of the secretion. It may be that such are ultramicroscopic. At the present time there are no anatomical signs which may be regarded with any degree of confidence as evidences of functional activity on the part of the parathyroids.

According to Ethel M. Luce (*Journ. Path. & Bact.*, 1923, 26, 200) a deficiency of calcium in the diet gives rise to hyperphasia of these bodies.

3. *Pituitary Body*

Pars anterior propria.—The pars anterior propria is made up of epithelial cells in clumps and columns with a small

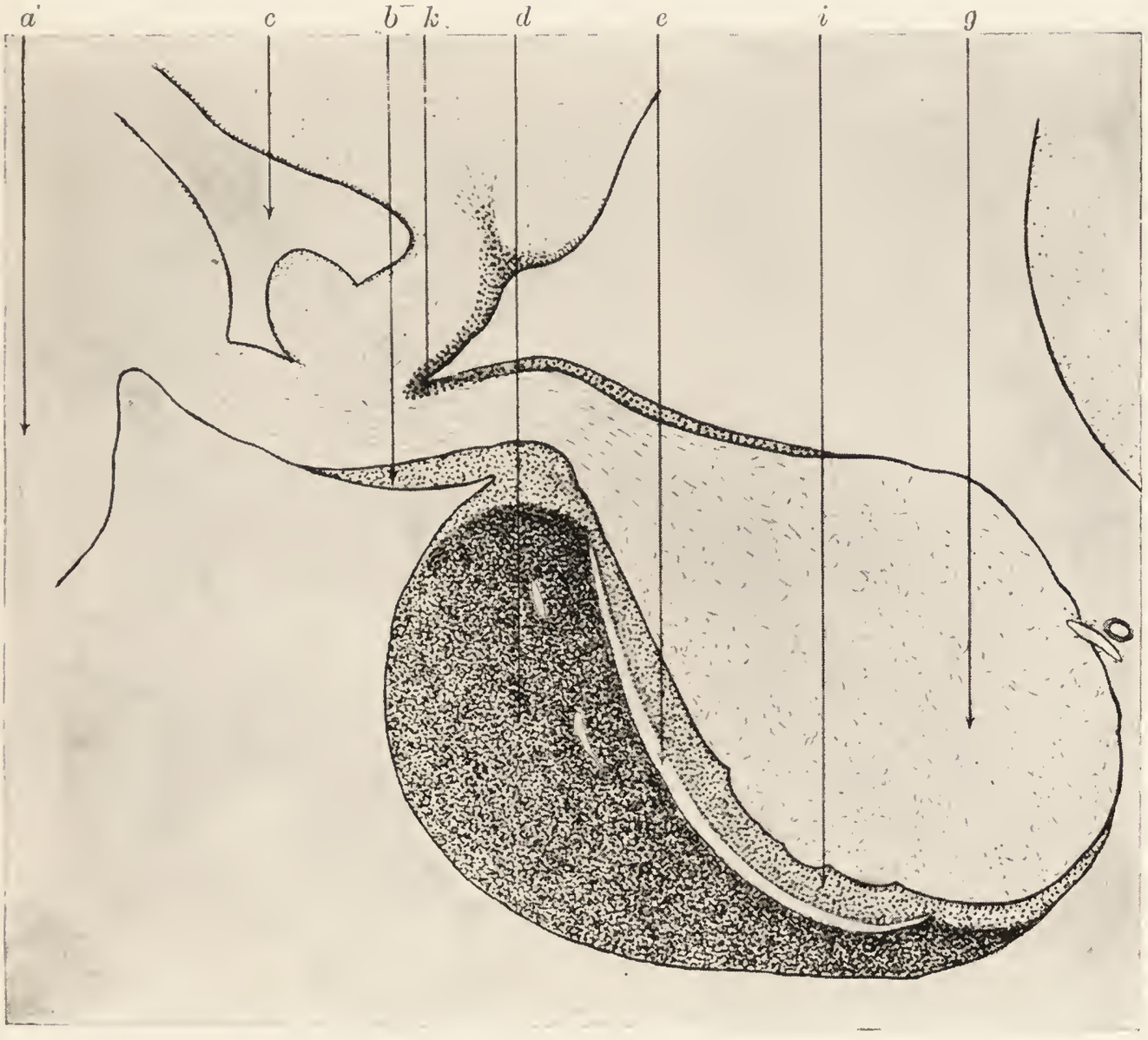


FIG. 23.—Mesial Sagittal Section through the Pituitary Body of an Adult Monkey.

a, optic chiasma; *b*, tongue-like process of pars intermedia; *c*, third ventricle; *d*, anterior lobe; *e*, epithelial cleft of posterior lobe; *k*, epithelium of pars intermedia extending over and into adjacent brain substance; the dark shading indicates anterior lobe proper; the lighter shading shows the position of the epithelium of pars intermedia; *g*, nervous substance of posterior lobe; *i*, epithelial investment. (Herring, from *Quarterly Journal of Experimental Physiology*.)

amount of connective tissue. From time to time acinus-like structures are met with and occasionally even lumina. The cluster arrangement is more marked in man than in other animals. The usual classification of the cells found in the glandular pituitary is as follows (see Figs. 23 and 24).

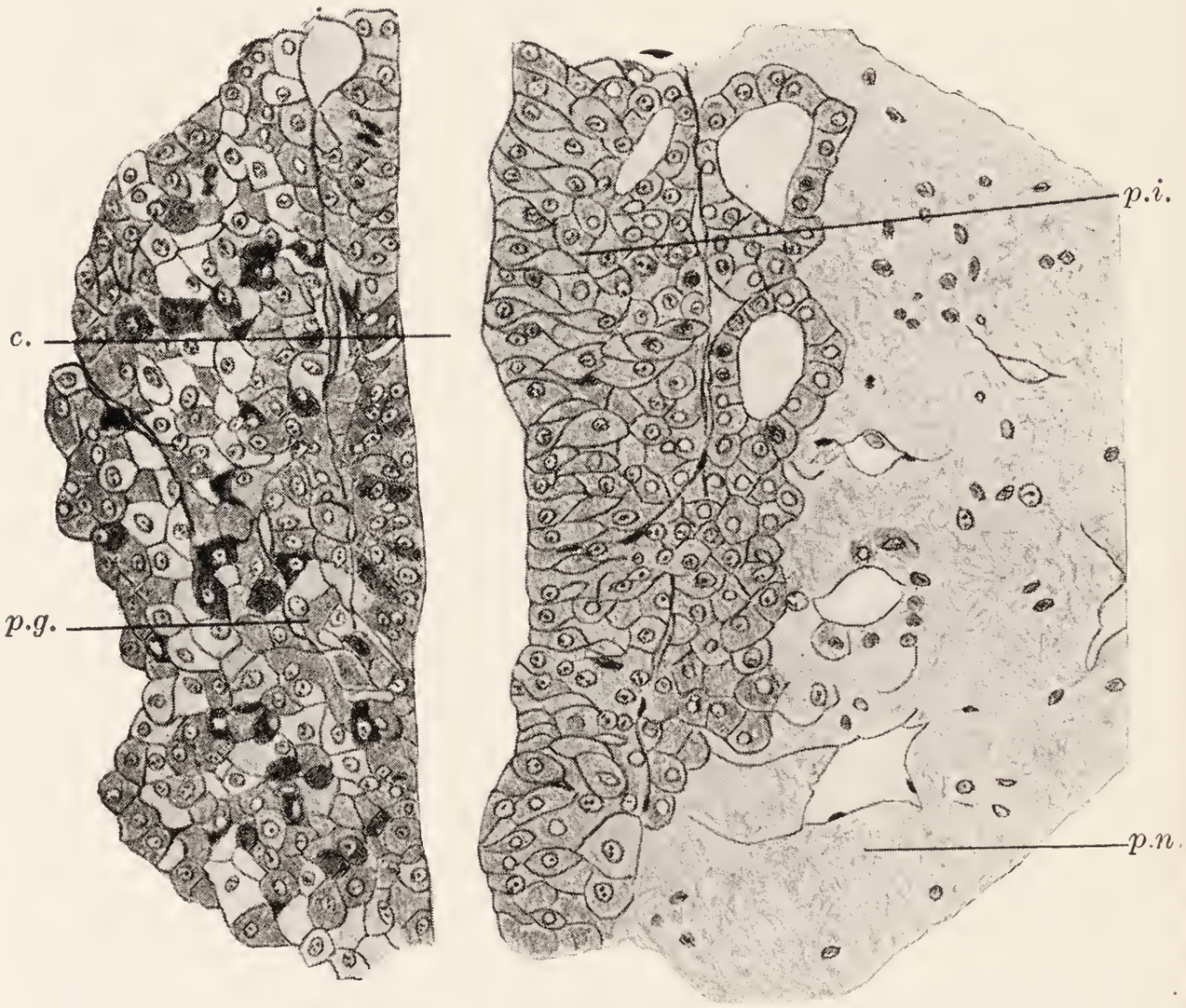


FIG. 24.—Section through portion of Pituitary Body of Dog, showing Glandular and Nervous Portions and pars intermedia.

c, cleft in glandular portion between glandular portion proper and pars intermedia; *p.g.* glandular portion showing three kinds of cells; *p.i.*, pars intermedia; *p.n.*, pars nervosa (Vincent).

1. Chromophile { (α) Acidophile. (β) Basophile.
2. Chromophobe.

The chromophile cells stain deeply by most ordinary methods, the chromophobe cells remain clear. In the chromophile cells there are two groups, those with acidophile cytoplasm and those containing basophile granules. The chromophobe

cells are often called the "chief" cells. There are apparent transitions between these different kinds of cell. It is not known whether the different kinds have different specific functions or whether they represent stages in a single type of activity. The acidophile cells are usually more abundant in the posterior part of the glandular portion.

It is believed by some observers that there is a definite secretory polarity in which the capillaries take the place of the ducts in ordinary secreting glands. This is deduced from the fact that a heaping up of granules occurs near the blood-vessels. Fat droplets are present in the cells and increase with age of the animal, as in the case of other glands.

There is nothing remarkable about the nuclei, the mitochondria, or the Golgi apparatus, which last forms a loose network. The acidophile granules are very conspicuous and may represent the true antecedents of the glandular secretion (Benda).

Various conditions produce effects on the cells of the pars anterior propria. In pregnancy the chief cells increase in number, becoming more abundant than the acidophile. They also increase in size and become slightly acidophile. After the end of pregnancy they return to the normal state. Castration also gives rise to an increase in number and size of the chief cells.

Thyroidectomy produces a deposit of hyaline substance in the pars anterior (Kojima). During hibernation the cells lose their characteristic staining reaction.

Pars intermedia.—In this portion of the organ the cells are arranged in groups with a variable amount of connective tissue between them. The cells are smaller than in the anterior lobe and contain fine neutrophile granules. It is said that they contain glycogen. Mitochondria are present, but the Golgi apparatus has not yet been seen. There is no secretory polarity, but granules of secretory antecedents have been described. The pars intermedia is less vascular than the pars anterior.

The presence of vesicles containing colloid in this portion of the organ is interesting and has given rise to much discussion. The colloid increases in amount after thyroidectomy and after castration in young animals. The significance

of this colloid material is not known. There seems to be a tendency to the formation of vesicles containing a colloid, or a substance resembling it, in many of the "ductless glands." It is stated by Simpson and Hunter that the "colloid" of the pituitary does not contain iodine.

Pars nervosa.—The nervous portion proper consists of neuroglia fibres and cells. Pigment is often present between the neuroglial elements. There are no appearances suggestive of glandular activity. Nevertheless "active" substances can be obtained from it, even when there can be no contamination with material from other portions of the gland (Vincent [121]).

4. *The Adrenal Body* (Cortex)

(See Figs. 25 and 26)

There is no need to describe the classical three layers of the adrenal cortex. They are of course most typical in the human subject, but it is surprising how constant is the general arrangement throughout mammals. It is still more surprising to find that the same three layers are found in the majority if not all the accessory cortical bodies.

Two types of cell are usually described in the adrenal cortex, the clear cells and the dark cells, appellations derived from their respective appearance in stained preparations. The clear cells (most abundant, at any rate in the guinea-pig) contain: (1) highly refractive spherical, fatty-looking droplets staining with Sudan III, blackening with osmic acid, and soluble in essential oils.

(2) Doubly-refracting lipoid globules.

According to Elliott and Tuckett [47] the former differ considerably in different species and may be absent in the sheep. Both kinds of droplets or globules disappear, of course, in the course of ordinary methods of preparation and are represented by vacuoles. Pigment is common in the zona reticularis. Typical mitochondria Golgi apparatus and trophospongia are found in these cells.

The dark cells stain deeply with iron hæmatoxylin, and hence are sometimes called "siderophile." The appear-

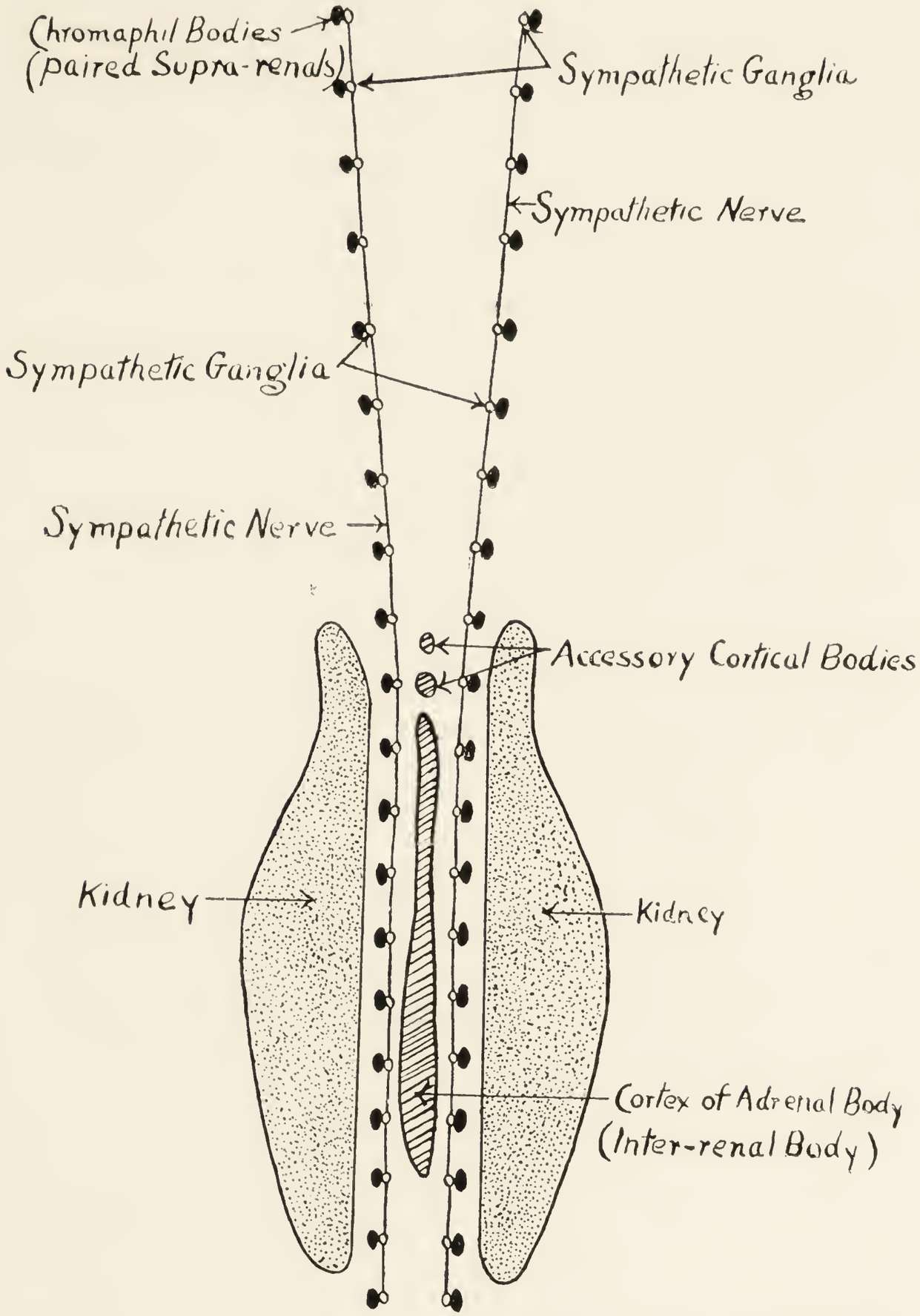


FIG. 25.—Diagram of the Adrenal Representatives in Elasmobranch Fishes, showing the Cortical Gland (Inter-renal Body) and the Medullary Glands (Chromaphil Bodies, "paired suprarenals") in relation to the Sympathetic and the Kidneys. (Vincent.)

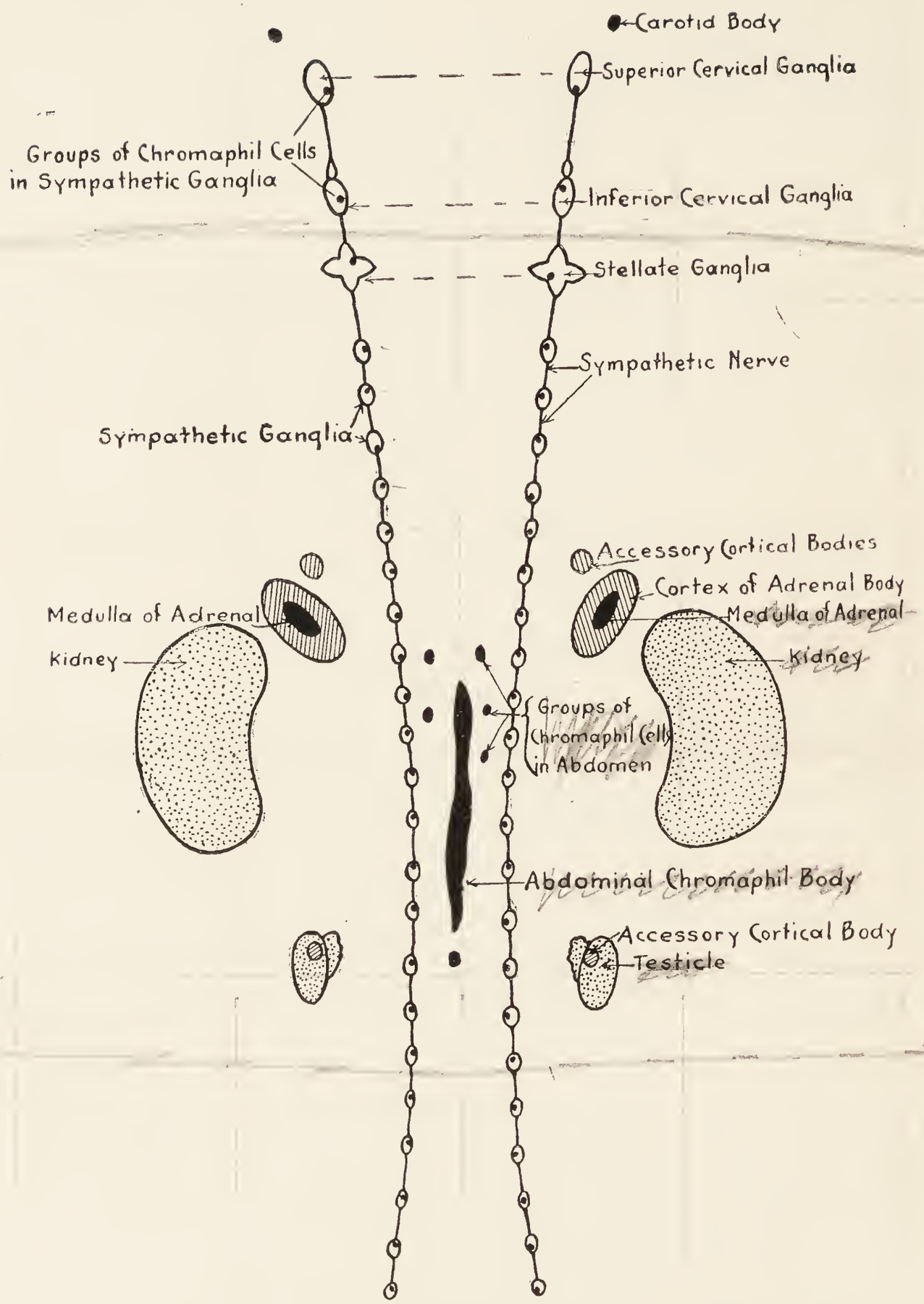


FIG. 26.—Diagram of the Adrenal Constituents and outstanding "Cortical" and "Medullary" (Chromaphil) Bodies in the Mammals, showing the Adrenal Bodies, the Chromaphil Cells of the Sympathetic, the Abdominal Chromaphil Body ("Accessory medullary") and Accessory Cortical Adrenals in relation to the Sympathetic and the Kidneys. (Vincent.)

ances in the cell due to the reaction with the iron stain are referred to as the "siderophile body." It is now considered that this structure is an artefact, but Goormaghtigh [63] considers that it is concerned in the secretory cycle.

Transitions between the two forms of cells may be found. It seems likely that they represent different stages in the activity of same kind of cell.

As to which of the cell contents is to be regarded as the true forerunner of the secretion, there has been much discussion. Most writers seem to favour the doubly refractive lipoid granules. The question also arises as to whether the lipoid globules are formed by the cortical cells or whether they are only deposited there and formed elsewhere, as in the "glandular adipose tissue"—the "lipoid gland" which surrounds the adrenal in many animals (Cramer). Da Costa expresses the opinion that the mitochondria are changed into lipoid. Mulon's view is that the mitochondria themselves are the secretory antecedents of a lipoid substance which impregnates the siderophile cells and passes through the intercellular spaces into the blood-vessels. He thinks that the interstitial cells of ovary and testis also produce a lipoid secretion. It has even been suggested that choline is the specific secretion of the adrenal cortex. Stilling, in his researches upon the adrenal body of the rabbit, observed periodic variations in the weight of these organs. There was enlargement of the glands in male animals during the breeding season. In the same communication he reports that the peripheral part of the cortex in the frog contains, during the summer, certain peculiar elements, the "summer cells," which atrophy later on during the pairing season. Patzelt and Kubik [103] have, however, come to the conclusion that Stilling's summer cells are present the whole year round, and are independent of age, sex or state of nutrition. These authors prefer to call the cells in question acidophile cells from their staining reaction. A curious point is brought out by these writers. They find that the acidophile cells are only present in one species, viz. in *R. esculenta*. They are entirely wanting in the adrenal bodies of the other anura which they investigated, as also from the glands of the urodela and the reptilia. They note also that similar cells are found

in the parathyroids of mammals and in the pituitary throughout vertebrates.

5. *The Chromaphil Tissues*

The microscopical appearances of the cells in the adrenal medulla have received a great amount of attention. This has been particularly the case since the discovery by Oliver and Schäfer of the blood-pressure raising principle which can be extracted from them.

The cells of the medulla are arranged, sometimes fairly regularly, sometimes quite irregularly, into columns and clumps, separated by connective tissue, blood-vessels, lymphatics and nerves. By ordinary modes of preparation the cell outlines are not so distinct as those of the cortex, and the granules have a great affinity for nuclear stains, such as hæmatoxylin, safranin, etc. The cell granules also give a characteristic reaction with ferric chloride. When moistened with a drop of a solution of this iron salt they turn green. The chromaphil reaction is the best known. This consists of a dark brown coloration when the cells are treated with a solution of the salts of chromium. This test indicates the presence of adrenin or some closely related substance. There appears to be different shades of colour according to time during which the reagent has acted. The oxidation products of adrenin are not yet known. It seems probable that the reaction occurs both with the cell granules and the ground substance. Macallum has called attention to the observation of Laignel Lavartine, that certain granules in the chromaphil cells reduce silver nitrate, and thinks that the reduction is probably due to the presence of tyrosin, an antecedent of adrenin. Macallum gets a similar reaction in nerve-cells and thinks they produce a certain small amount of adrenin. The osmic acid reaction will be referred to presently.

The extra-adrenal chromaphil bodies occur in the sympathetic ganglia and in various parts of the abdomen. The chromaphil cells of the sympathetic ganglia are arranged in groups and not divided up into distinct columns and smaller masses, as is the case with the abdominal chromaphil body

and the adrenal medulla. The cell-outlines are for the most part distinctly seen and are indicated in many cases by a light (unstained) ring, which is again surrounded by a dark slightly irregular border. The cells are about 15μ in diameter, and the protoplasm is very finely granular. The brown tint varies in intensity in different cells and in different parts of the same cell. The nuclei are large in proportion to the dimensions of the cells, and are on an average 7μ in diameter. The majority are stained brown by the bichromate, and the outlines are distinct and dark brown while the interior is usually clearer. There is sometimes a distinct nucleolus, and the outer portion of the nucleus is studded with small brown granules.

In the ordinary laboratory animals the extra-adrenal chromaphil tissues in the abdomen are now well-known. In order to show them the liver and alimentary tract are removed from the abdomen and a piece of absorbent cotton soaked in a solution of potassium bichromate (3·5 per cent.) is placed over the retroperitoneal tissues and left *in situ* for six to twelve hours. At the end of this (or in some cases a longer or a shorter time) the adrenal bodies, aorta, vena cava, and subjacent and superjacent tissues are cut out as far back as the bifurcation of the aorta and washed in running water for several hours. The chromaphil bodies are then plainly seen, and still more plainly if the whole preparation be placed in glycerine. They appear as dark brown streaks, patches, or dots of varying size and shape.

In the dog, the extra-adrenal chromaphil tissues are abundant, and the "abdominal chromaphil body" is easily and beautifully shown by the method described above (see Figs. 27 and 28). A few minutes after the mop of absorbent cotton soaked in bichromate has been placed over the aortic region, a long brown ribbon of rapidly deepening tint may be seen lying over the aorta, or slightly to one or the other side. In from half an hour to an hour its deep brown stain may be fully developed. It measures four or five centimetres in length and may reach a width of 5 mm. Numerous irregularly placed small nodules of chromaphil tissue are seen in different regions near the principal body.

In preparations made with bichromate solutions the chroma-

phil cells are stained of a tint which varies from a light yellow to a deep brown, according to the duration of the treatment with the solution, and apparently also according to the physiological conditions in the cells. A transverse section is seen to be divided up into a number of irregular areas which represent the cross-sections of columns of cells which run longitudinally. The width of these columns varies considerably. They are divided from each other by strands of connective tissue with a large amount of elastic tissue and by blood-vessels and nerves. In the spaces between the columns is also accumulated a quantity of homogeneously stained material which superficially, at any rate,

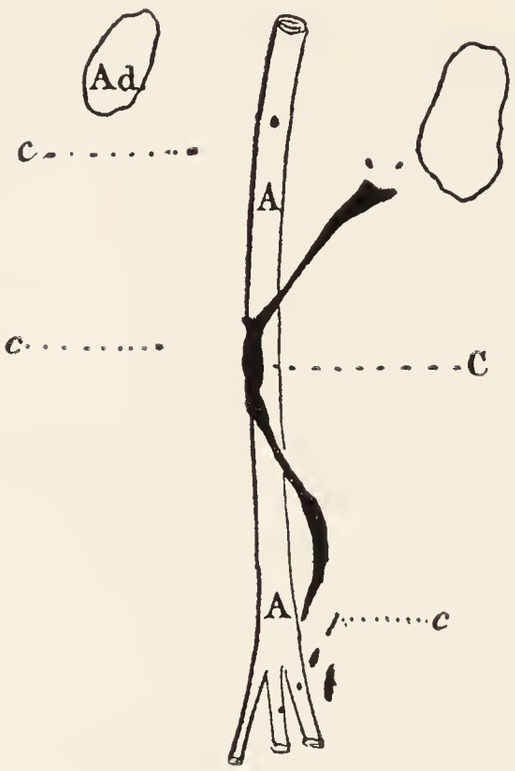


FIG. 27.—Abdominal Chromaphil Body of an adult Dog.

A, aorta; Ad, adrenal; C, abdominal chromaphil body; *c*, smaller chromaphil bodies. (Vincent.)

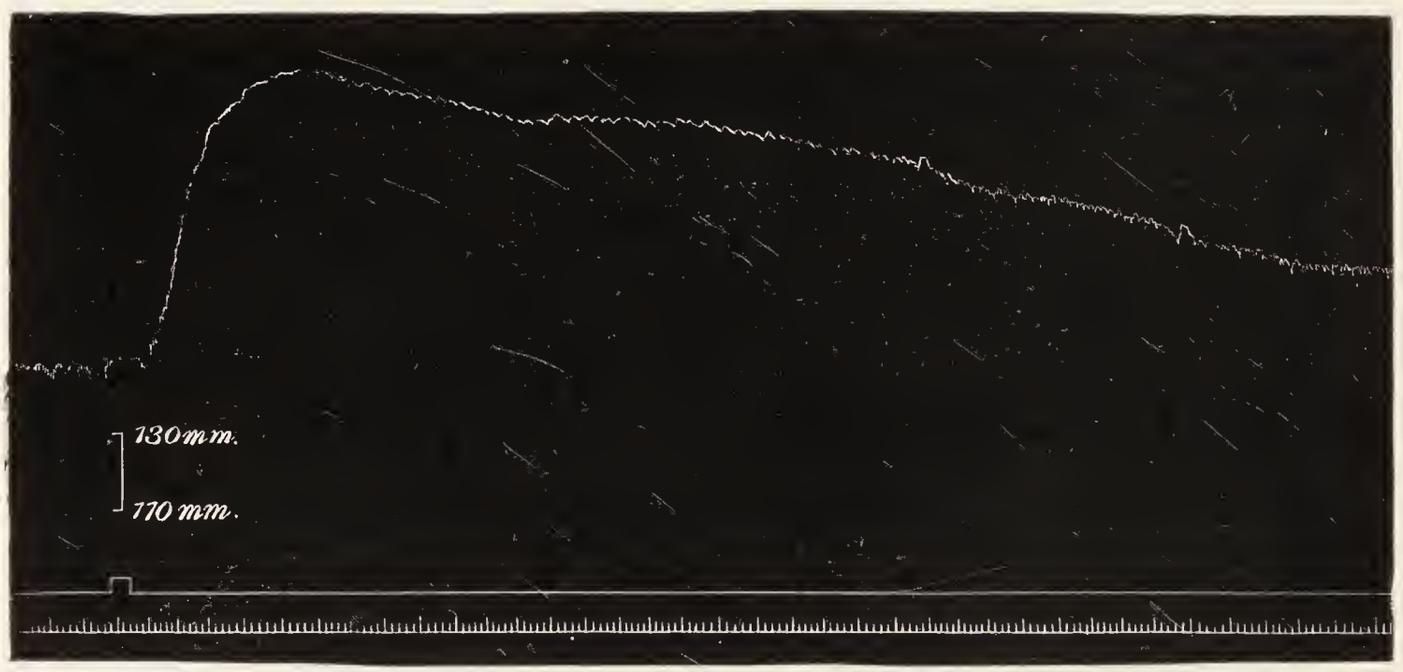


FIG. 28.—Tracing showing Effect of an Extract from the Chromaphil Bodies of three Dogs when injected into the Saphenous Vein.

appears to resemble the colloid of the thyroid. This seems to be in some instances contained in lymph spaces.

The cells are irregular in shape, but tend, like those of the chromaphil groups in the sympathetic ganglia, to be circular in outline. They are slightly smaller than those in the ganglia. The protoplasm is very finely granular or almost homogeneous, and the intensity of the brown coloration varies considerably in different cells. In all parts there are clear spaces containing a substance like colloid above referred to. The nuclei resemble those of the chromaphil cells in the ganglia.

The general resemblance between extra-adrenal chromaphil tissue and adrenal medulla is very great. Both consist of columns of cells staining yellow or brown with bichromate of potash. The cell-columns are, however, for the most part much thicker in the adrenal than in the abdominal chromaphil bodies. The blood-spaces are wider, and the whole aspect gives the impression that the adrenal medulla is more highly organized. Many of the cells of the adrenal medulla are spherical, as in the abdominal chromaphil body, and their dimensions are the same. The nuclei, also, are of the same order of magnitude in both structures. But in many regions, especially where the cell-columns are separated by large venous sinuses, the cells are arranged in a definitely epithelial fashion round the blood-vessels. In this case the cells are columnar and the nuclei are placed at the end of the cell remote from the blood-vessel (Vincent [121] [120]).

The protoplasm of the adrenal medulla is perhaps more distinctly granular than that of the abdominal chromaphil body, and is, moreover, more delicate in consistence, and therefore tends to show greater shrinkage in the processes of fixation and tearing during the process of cutting sections. These considerations serve to explain many of the unsatisfactory drawings and descriptions of the earlier workers. When the adrenal body is fixed in bichromate solutions, the section shows vacuoles similar to those described above in the chromaphil body. These are absent in sublimate and Flemming preparations (Vincent [121] [120]).

Thus it seems justifiable to regard the medulla of the adrenal body as composed of chromaphil cells of the same general character as those forming the chromaphil bodies. But

the former have undergone specialization, and the structure of the substance has become elaborated into an organ with more definitely glandular form. The abdominal chromaphil body gives the physiological test for adrenin.

Histological Evidence. We have now to consider the histological evidences of processes of secretion in the adrenal medulla. Secretory changes in this structure have been described by many authors. Manasse gave a description of brown masses in the blood-vessels of the adrenal body, and small, highly refractive, colourless granules in the adrenal vein of the dog, and other observers have found in the blood taken from the adrenal vein a number of highly refractive granules. Carlier many years ago called attention to deeply staining granules in the adrenal medulla of the hedgehog. These he found also in the lumina of the venous sinuses either singly or in clumps, and states that they could be observed in different stages of elimination from the cells. Stilling says that in " hunger " frogs the medullary cells are vacuolated, and take on the bichromate reaction in an irregular fashion. In summer frogs there is a distinct reduction in the amount of the medullary substance. It is said that following prolonged narcosis in man with the accompanying discharge of adrenine, the chromaphil reaction is reduced, and that the same occurs after Bernard's " piqûre " of the medulla oblongata. Ether is usually supposed to cause a discharge of adrenine and reduction of the chromaphil reactions in experimental animals.

Hultgren and Andersson give a description of certain characteristic granules in the cells of the adrenal medulla, and also in the blood of the adrenal vein. They think that the passage of these through the endothelium of the blood-vessels can be observed. Similar granules both in cells and veins have been described by numerous observers, and some have even claimed to have recognized these as adrenine by their micro-chemical reactions. Félicine and also Ciaccio have described intercellular canals, and the latter states that the specific granules are of two kinds : the one having a special affinity for the salts of chromic acid—the chromaphil granules ; the other having a special affinity for perchloride of iron.

Stoerk and Haberer [117] are of opinion that the true secretion is not granular, and that granules do not pass through the cell membrane. This, of course, is a possible criticism of all histological methods of studying secretion. Even if the actual granules are artefacts they may still give us valuable information about the changes in the cells. The authors in question regard the granules as representing structural units which are possibly to be regarded as the seats of chemical action whose products are to be looked upon as the true secretory materials of the medullary cells, which materials pass into the blood by some such process as diffusion. The fluid secretory product is the true bearer of the chromaphil reaction of the medullary cells, the granules giving the reaction only in their secretory phase, when they are just forming the chromaphil substance. The fluid secretion can be recognized intracellularly (in both protoplasm and nucleus) and extracellularly (in admixture with the serum of the capillary and venous blood). Besides the typical fine granules of the medullary protoplasm, there are coarse structures which occur on the side of the cells turned towards the vessels, and which reveal a different staining reaction from the granules.

Cramer [36] has recently revived the old method of osmic vapour fixation and employed it in the study of the adrenal medulla. He finds that in the normal gland of the mouse most of the medullary cells are filled with very fine granules, which he thinks are adrenine. These are stained a dull black like coal-dust and are evenly distributed throughout the cell. The nuclei show a clear karyoplasm with the network of chromatin stained grey. But there are also a large number of clearer cells which are mostly arranged in round "alveoli," giving the appearance of islets. These cells, which have a swollen appearance, are filled with very fine granules stained a light grey and contain in addition one or more round black "globoid bodies" or "spheroids" of varying size which are irregularly placed within the cell. They may be attached to the nuclear membrane or to the cell wall. Sometimes they can be seen lying outside the cell altogether in the narrow intercellular spaces. These globoid bodies have a superficial resemblance, especially

in size, to the lipoid globules of the cortex of the organ. But on closer inspection a distinct difference in the quality of the black stain can be noted, and moreover they are not soluble in turpentine as are the lipoids and fats (see Figs. 29–32).

These islets of clearer cells are probably not, in Cramer's

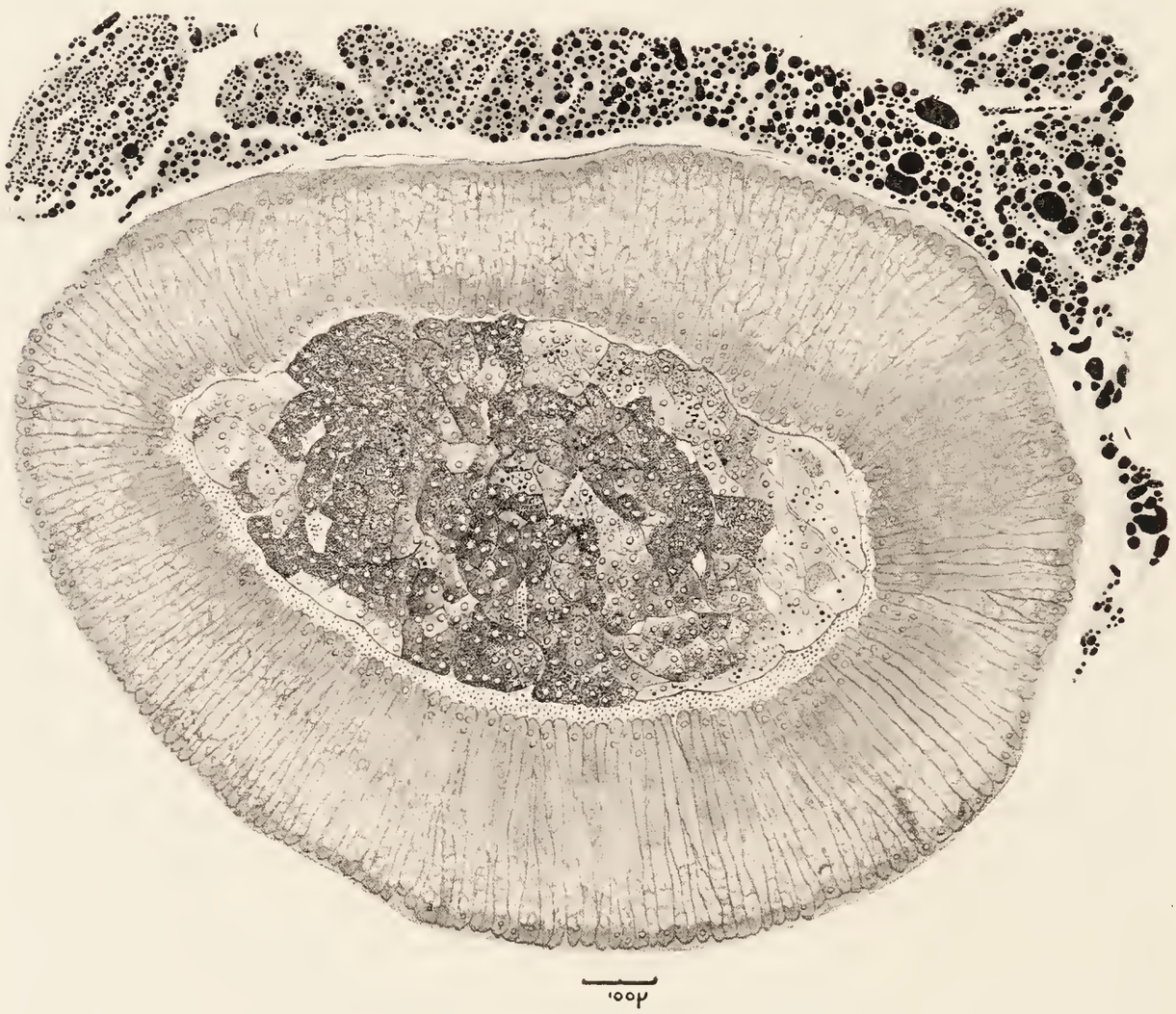


Fig. 29.—Suprarenal Gland of normal Mouse, fixed in Osmic Vapour.

The lipoid granules of the cortex have been dissolved out in the Canada balsam, and are represented by vacuoles. The figure shows the sharp separation of medulla and cortex. In the medulla note two distinct appearances shown in the medullary cells by this method: cells uniformly filled with fine black granules and clear cells with larger black spheroids. The zona reticularis is narrow. The peri-adrenal adipose tissue has retained the osmic stained fat. Low power, × $\frac{60}{1}$. (Cramer.)

view, a tissue *sui generis* but represent resting medullary cells in a special stage of functional activity, probably that of a cell which has secreted its content of adrenine and is about to refill itself again. This is to be inferred from the fact that the islets are more numerous in a gland which has been active, and that sometimes these islets are clear, while at other times they are filled with very fine granules which

give them a greyish appearance, but they still contain the black spheroids. In the medulla of the normal mouse no, or very few, free "adrenine" granules are seen in the large central vein or its tributaries.

FIG. 30.—Exhausted Suprarenal of a Mouse dying as the Result of Exposure to Wet and Cold for 4 hours.

The cortical lipoids are not obviously diminished, and are represented here also by vacuoles. The zone reticularis is swollen and broadened, and many of its cells contain small black globules. The medulla is completely exhausted, as shown by the absence of fine granules. A number of clear cells still contain black spheroids. Low power, × $\frac{60}{1}$. (Cramer.)

Cramer finds these islets of clear cells also in the adrenal body of the rat, the cat and the dog.

After a large dose of beta-tetrahydronaphthylamine the central vein is found to be filled with black "adrenine" granules. Many of the medullary cells have lost their cell outlines, and their cytoplasm gives the appearance of having become "laked." In such cells the "adrenine" granules are no longer visible, the cytoplasm being stained an even

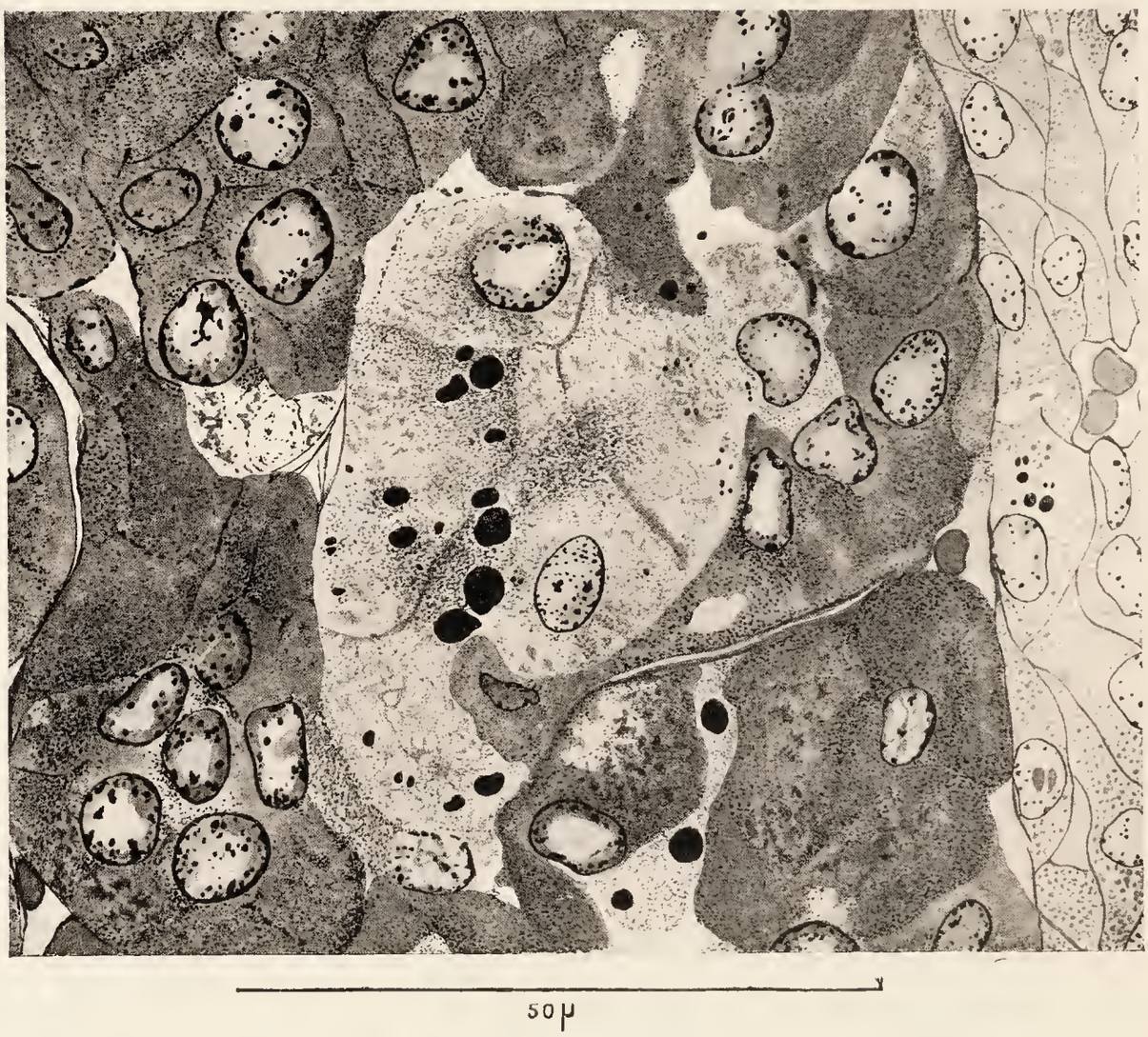


FIG. 31.—Suprarenal of a Mouse which remained well after Exposure to Cold overnight for 9 consecutive Nights and was then killed.

Surface of medulla with a few layers of the swollen zona reticularis (on right). One of the cells of this zone contains a number of small black globules, which are not fats or lipoids. The figure shows a clear island in the medulla with black spheroids, surrounded by fully-loaded medullary cells. The appearance of the medullary cells in this figure is practically identical with that of a normal "resting" gland. High power, × $\frac{1}{1}\frac{1}{6}\frac{0}{0}$. (Cramer.)

dark brown. Their nuclei have shrunk, the clear karyoplasm has disappeared, and the nuclei now appear as small dark brown elliptical bodies resembling blood corpuscles. Cells showing this appearance are distributed irregularly throughout the medulla. A similar state of affairs is brought about by exposure of the mouse to severe cold (see Figs. 29, 30, 31, 32).

Cramer reports that the above-described changes in the medullary cells are accompanied by changes in the cortex, particularly in the zona reticularis. This zone becomes deeply congested and the congestion extends to the medulla. The cells of the zona reticularis increase in size, so that the

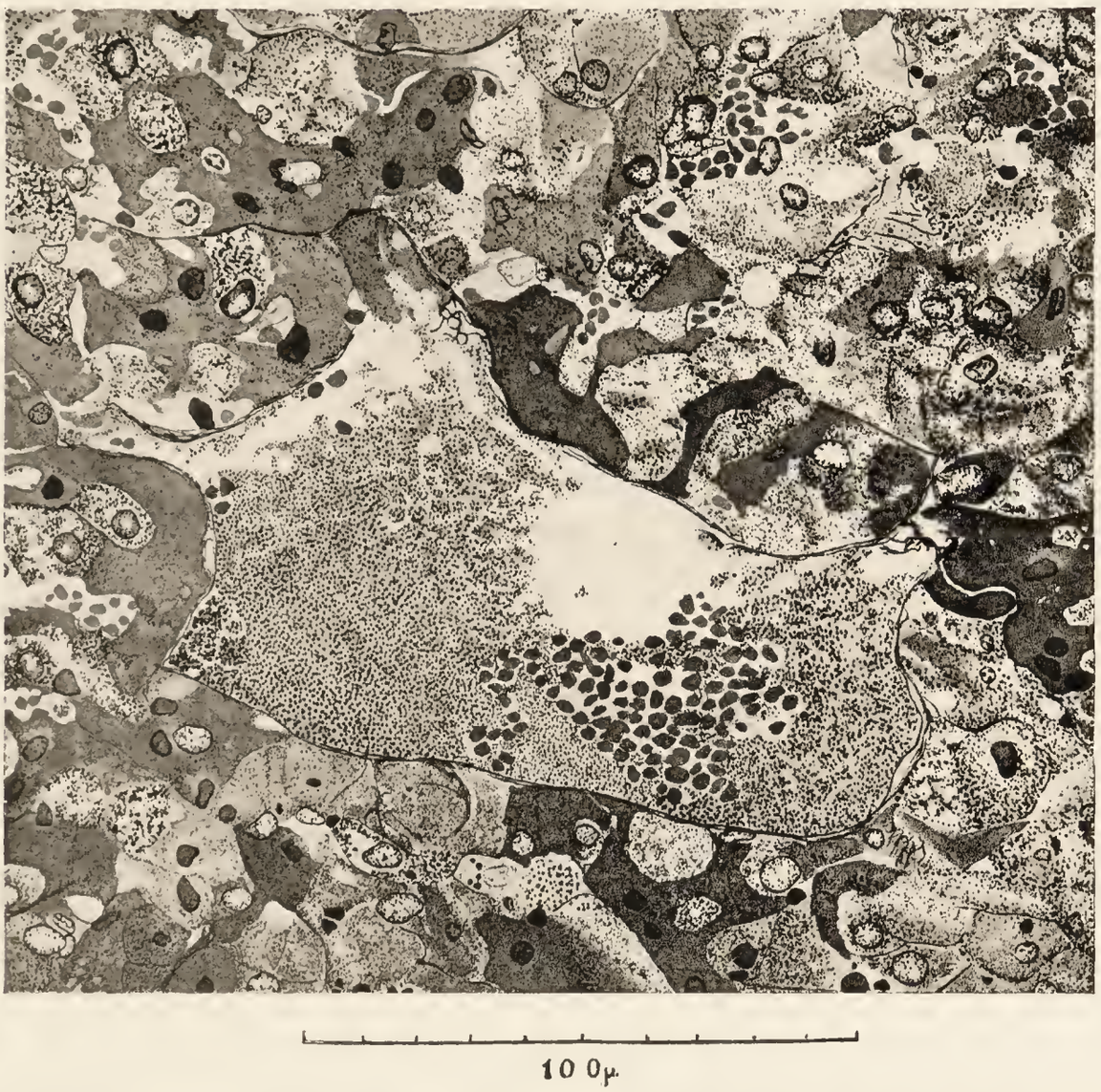


FIG. 32.—Suprarenal of a Mouse which died 40 minutes after Injection of 2·5 mg. T.H.N.

The gland was swollen and congested. In the centre of the figure there is a vein nearly filled with fine osmic-blackened granules of adrenalin. Most of the cells are extensively vacuolated and contain adrenalin granules. These also occur in the intercellular spaces. Immediately bordering on the vein wall there are very darkly-stained "laked" cells with shrunken glazed nuclei. High power, × $\frac{480}{1}$. (Cramer.)

zone as a whole expands and instead of a narrow thread now appears as a broad band. Moreover, in these cells, according to Cramer, may now be seen small globules staining black with osmic vapour which are not fat or lipoids. Without deciding that these represent adrenine or a precursor, Cramer concludes from their occurrence in the various conditions

demanding increased activity of the glands, that the cortex takes part in the functional activity of the medulla, and that these two parts of the gland are not two physiologically independent organs. These observations, if confirmed, have a very important bearing on the question of the relationship between cortex and medulla of the adrenal bodies. Up to the present time there has been no evidence worth considering that the two portions of the gland have any relation to each other, either embryological or physiological. Indeed Cramer's hypothesis presents many difficulties from the morphological standpoint. Any functional relationship between cortex and medulla can scarcely hold in the elasmobranch fishes where the "interrenal system" and the "chromaphil system" have no anatomical connections with each other. But since Cramer postulates a temperature regulating function for the compound mammalian gland (in conjunction with the thyroid) he would probably admit that in the cold-blooded animals the service of cortical and medullary representatives in the economy might be something quite different.* Moreover, even if it could be shown conclusively that under certain conditions adrenin granules are found in the part of the cortex next to the medulla, this would by no means prove that the cortex normally takes a part in the production of adrenin. It might be supposed that under pathological conditions adrenine might not be able to escape by the usual channels, and so would overflow into the neighbouring cortex.

Cramer reports that depletion of the adrenal medulla occurs in acidosis, after hæmorrhage and anæsthesia, as well as after bacterial infections.

I have reported these investigations of Cramer in some detail, because there can be little doubt about the actual appearances found, and the changes occurring, for example, as a result of exposure to cold. But it must be urged that, although these changes constitute the best histological evidence of secretion in the adrenal medulla, more evidence

* It must be admitted that the gradual coming together of the adrenal and certain chromaphil cells in the course of phylogeny may not be without significance. A new function may have been assumed along with the new anatomical relations.

is required before we can admit these appearances as proof of a true normal secretory process.

6. *The Islets of Langerhans* (see Fig. 33)

Since the work of Bensley[16] it has been generally recognized that the cells of the islets are not all of the same kind.

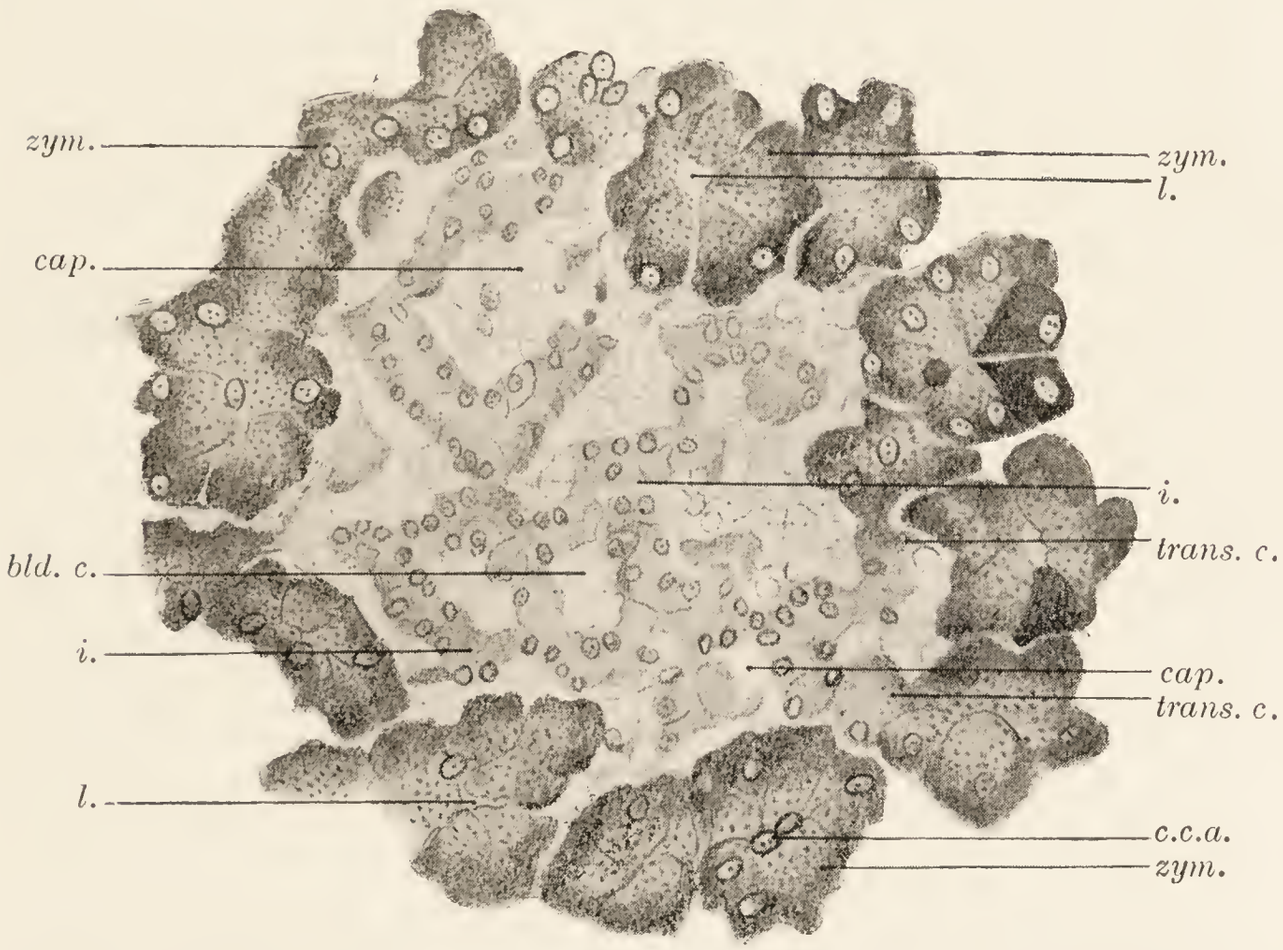


FIG. 33.—Islet of Langerhans from the Splenic End of the Pancreas of a normal Dog.

bld.c., red blood corpuscles ; *c.c.a.*, centro-acinar cells ; *cap.*, blood capillaries ; *i.*, islet of Langerhans ; *l*, lumen ; *trans.c.*, transitional cells ; *zym*, zymogenous tissue. (Vincent.)

In the guinea-pig three varieties are described : the A cells ; the B cells, and the "indifferent cells."

The B cells are the most numerous. They contain granules which, after fixation in the acetic-osmic bichromate mixture, are basophile, staining with methyl green. Mitochondria are found, sometimes accumulated in the part of the cell next to the blood sinus. The Golgi apparatus is usually at the opposite pole of the cell. This may indicate a secretory polarity (Cowdry). These cells are supposed by some

observers to be the elements which are more important from a physiological standpoint.

The A cells are fewer in number and contain granules which stain with fuchsine instead of with methyl green after acetic-osmic bichromate fixation.

The "indifferent" cells appear quite clear and are much fewer in number. It is thought that they are the parent tissue, from which the A and B cells arise.

These three types of cell are said to occur in man, several laboratory animals, and the toad.

Laguesse and the present writer among others have recorded that the islets are increased in inanition, but the majority of writers have accepted Bensley's views that such changes do not occur. The usual belief that the islets are organs *sui generis* and are anatomically independent of the zymogenous tissue is based upon somewhat insecure foundations.

Thyroidectomy is said to produce an increase in the amount of islet tissue. According to Kojima feeding rats with posterior lobe of pituitary causes the cells of the islets to stain more deeply with Mallory's stain, on account of the accumulation in them of granules possessing an affinity for Orange G. Homans [73] states that he produced various degrees of diabetes in the dog by removal of portions of the pancreas, and found that the mitochondria in the B cells became first accentuated and ultimately disappeared, while those in the other cells suffered no change. He thinks that it is the B cells which assist in the processes of carbohydrate metabolism, and that the changes in the cell are those of over-activity followed by fatigue and exhaustion. As pointed out by Cowdry [35] the alterations may result from the diabetes, or in other words, the B cells may be merely peculiarly sensitive to the altered conditions.

Nothing definite is known of any changes in granules representing different stages of secretory activity.

7. *The Interstitial Cells of the Testis*

The interstitial cells are disposed in variable number between the seminiferous tubules. It is pointed out that they are often grouped about the blood-vessels in a manner which

suggests a process of internal secretion. The cells develop very early in fœtal life even before the formation of definitive germ cells. They are throughout life very liable to form tumours.

The interstitial cells occur in most vertebrates, but their degree of development varies within very wide limits. Rasmussen states that in hibernating animals they undergo cyclical changes in number. During the hibernating period they are reduced.

The cells appear to present the usual features of gland cells. Mitochondria, centrosomes, fat droplets, pigment, Golgi apparatus, and various cell inclusions have been described. The secretory products are generally supposed to be of a lipoid nature, though definite changes in different stages of activity have not yet been proved to occur.

8. *The Interstitial Cells of the Ovary*

The distribution of the interstitial cells of the ovary is like that of those of the testis, extremely variable throughout vertebrates.

The cytoplasm of the cells is filled with lipoid droplets, and it is usual to believe that the elements produce a lipoid secretion in common with those of the testis and the adrenal cortex. The lipoid granules are generally regarded as the secretions antecedent. Nothing is known of any changes in these granules or of their discharge into the blood-current. (For a fuller account, with references to literature, see Cowdry [34].)

9. *The Corpus Luteum* (see Fig. 34)

Secretory changes in the corpus luteum have been described by Van der Stricht [118] in the bat. This author gives an account of histological changes which give two kinds of secretion, a serous and a lipoid. The former resembles the liquor folliculi and is formed, after the rupture of the follicle, by the young lutein cells. It is produced in the form of minute drops within the cells, and is poured out into the intercellular spaces and the lymphatics. This secretion increases during the early segmentation of the ovary, decreases later, and finally

disappears when the ovum reaches the uterus. In the opinion of Van der Stricht the secretion exerts some directive influence upon the uterine changes.

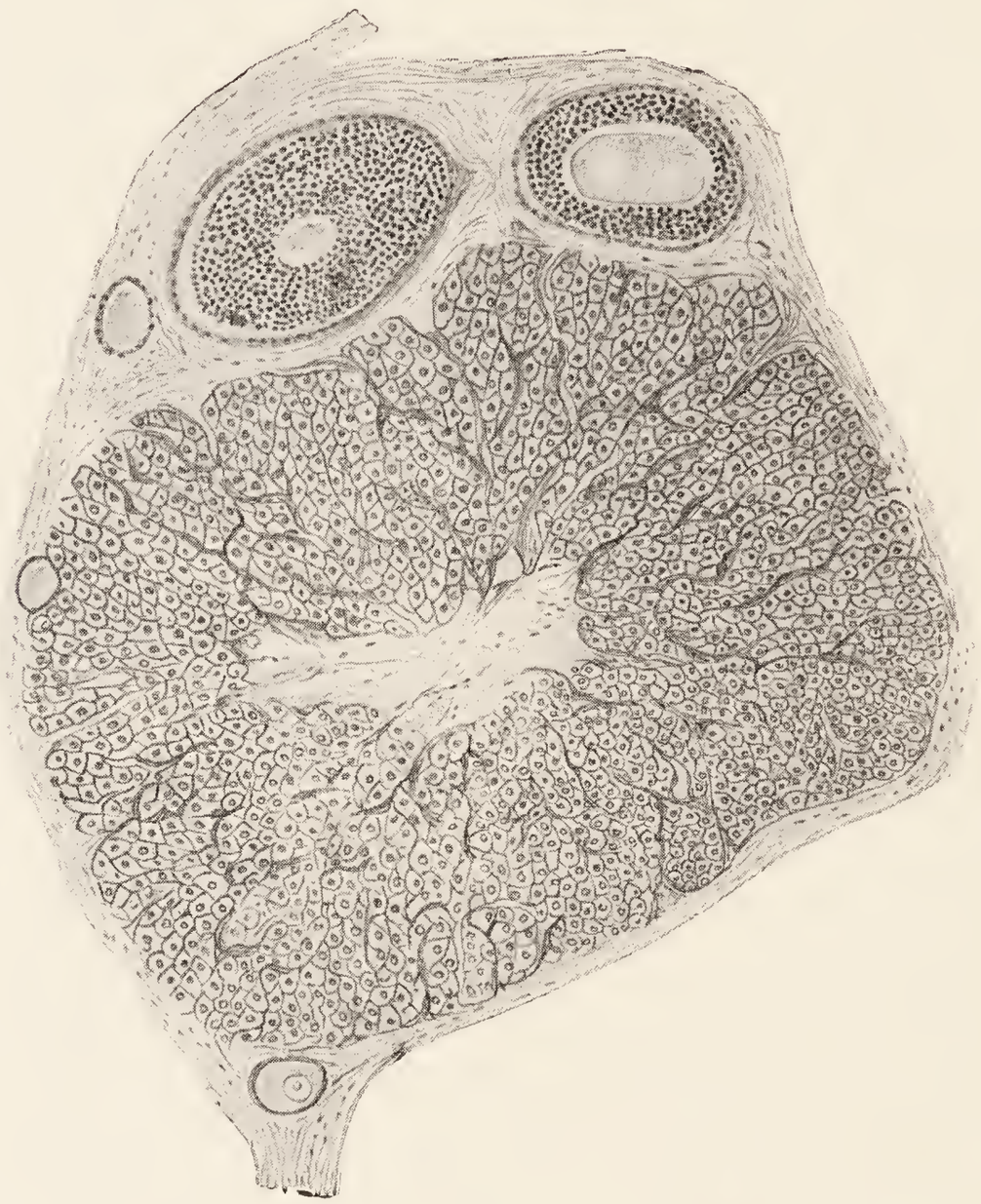


Fig. 34.—Section through the Ovary of *Dasyurus viverrinus*, showing Graafian Follicles and Corpus luteum. (Vincent.)

The lipoid secretion is derived from fat in the lutein cells, and is excreted into the intercellular spaces and lymphatics. It serves to fix the ovum and aid the formation of the placenta.

CHAPTER VIII

THE EXPERIMENTAL AND CLINICAL EVIDENCES FOR "INTERNAL SECRETION"

1. *Introduction*

In the majority of instances the hypothesis that any particular organ or tissue furnishes an internal secretion has not been based upon histological evidences of secretion but upon the results of disease, extirpation, injection of extracts, etc. We have now to examine the data so derived in regard to each of the organs and tissues in question.

2. *The Thyroid Body*

It is found that removal of the thyroid in young animals (one or more parathyroids being left behind) will bring about a condition resembling cretinism in the human subject. In older animals symptoms may not be observed. But occasionally there may be thickening and dryness of the skin and other symptoms. Myxœdema, as it is found in the human subject, does not occur in animals.

When the thyroid is atrophied in the human subject we get the condition known as myxœdema, which may be remedied by the administration of extracts made from the thyroid. The active principle is now generally considered to be Kendall's "thyroxin."

Extracts of thyroid and "thyroxin" itself have a remarkable effect in increasing the metabolism of the body.

Nothing is known of the thyroid secretion in the blood, and extracts of thyroid do not contain any active principles which produce any very striking immediate effects upon circulation, respiration, secretion, etc. There is some evidence that the pouring out of the active substance from the gland is under

the control of the sympathetic nervous system. It is said that electrical changes may be observed in the thyroid during activity.

It will thus be seen that in the case of the thyroid we have the essential criteria of internal secretion except the presence of the active substance in the blood.

3. *The Parathyroid Body*

Removal of the parathyroid glands usually produces serious symptoms of "tetany" and frequently death ensues. In some animals, however, it is possible to remove all four bodies without inducing any serious symptoms. The parathyroids have frequently been removed, along with the thyroid in the human subject without any ill effects.

Many writers believe that "tetany" in the human subject is due to disease of the parathyroids and that treatment with extracts of parathyroid is valuable.

It is now generally supposed that the organs manufacture some material which either destroys certain toxic products of metabolism or renders them inert. According to Paton "the parathyroids control the metabolism of guanidin in the body by preventing its development in undue amounts." Some authors believe that they are concerned with calcium metabolism.

4. *The Pituitary Body*

It is generally considered that total extirpation of the pituitary body is a fatal operation, though the duration of life in the operated animal varies very considerably according to its age. Young animals live longer than old ones. Partial extirpation (if a small part of the anterior lobe be left) gives rise to disturbances in growth and metabolism, and these may be induced in young animals by deficiency of anterior lobe only. The effects upon the genital organs may be due to deficiency of the pars intermedia, and the preponderance of evidence is that polyuria and disturbance of carbohydrate metabolism (sugar tolerance) are due to deficiency of this part as well. Whether removal of the nervous portion only would entail serious results is not known. It is worthy of

note that, just as in the case of the adrenal body, so we have in the pituitary two main portions, one of them "glandular," the other nervous. In the case of both organs, the physiologically active substances are found in the nervous portion (neuro-hypophysis, adrenal chromaphil tissue), while the "glandular" portion is that which is essential to life.

But the recent work of Camus and Roussy [31] and of Bailey and Bremer [8] indicates that many, if not all, of the results usually attributed to lesions of the pituitary body are really due to damage to the base of the brain. These authors believe that the polyuria which occurs on extirpation of the pituitary is not due to removal of this organ, but to a lesion of the opto-peduncular region. This region lies at the level of the grey substance of the tuber cinereum in the vicinity of the infundibulum. This zone seems to play some part in the mechanism of water retention in the organism. Atrophy of the genital organs and the "adiposo-genital syndrome" are likewise due not to any hypophyseal lesion but to trouble at some point in the base of the brain. The same applies to the changes in tolerance to carbohydrates and the appearance of alimentary glycosuria. These experiments, if the results are confirmed, will necessitate a reconsideration of our whole attitude in regard to the pituitary body.

In regard to the pituitary, as in other cases, there has been too great a hurry to assume the occurrence of processes of secretion, from data which may be, after all, only of pharmacodynamical interest. Camus and Roussy express themselves on this subject as follows: "It is true that these extracts produce a contraction of the uterus. Seeing, however, that the effect is procurable by extracts obtained from bulls and steers, to deduce from the action of the extract the part played by the normal gland in the living bull or steer, is a somewhat embarrassing problem."

Extracts from the posterior lobe of the pituitary body produce a powerful prolonged rise of blood-pressure by constricting peripheral arterioles. A depressor substance is also present, and we may get mixed effects. There is slowing of the pulse through an action on the heart. Respiration is slowed or stopped. There are effects on various involuntary

muscles, viz., those of uterus, bladder, intestine, stomach, and pupil. There are effects on the secretion of certain glands—mammary gland, kidney, stomach glands. It is doubtful, however, how far we are justified in concluding that these effects are an evidence of an internally secreting activity. We shall see that the same sort of discussion arises in the case of the adrenal body.

Dixon [43] has recently affirmed that samples of normal cerebro-spinal fluid of the dog show the chemical and physiological actions of pituitary (posterior lobe) extracts. He finds that injection of ovarian extracts into the blood increases the physiological action of the cerebro-spinal fluid as tested on the isolated uterus of the virgin guinea-pig. From this he concludes that the pituitary " secretes " into the cerebro-spinal fluid. The chemical actions given are not sufficient to identify any substance. The effect of ovarian extract on the pharmaco-dynamical properties of the cerebro-spinal fluid is very striking in Dixon's tracing. Nevertheless great care has to be exercised in interpreting the results obtained by the study of isolated smooth muscle preparations.

5. *The Adrenal Body* (*Cortex*)

It is now generally conceded that the adrenal cortex is essential to life, though some animals appear to resist the effects of extirpation for a long time. The orthodox method of dealing with these exceptional cases is to assume that the accessory cortical bodies necessarily left behind are sufficient to maintain life.

Extracts from the cortex of the organ give rise to no immediate specific effects when administered to an animal. An effect on the growth of the testes has been observed, when cortical extracts are administered along with the food to young growing animals.

Indirect evidence, clinical and pathological, points to some connection between the organ and the reproductive system. Tumours of the cortex are often associated with precocious sexual development in children, or loss of female characters in older individuals.

Thus it is clear that the evidences for any definite " secretion " are almost nil.

6. *The Chromaphil Tissues*

The mass of chromaphil tissue which constitutes what is called the "medulla" of the adrenal body has aroused universal attention, owing to the discovery by Oliver and Schäfer that the structure yields to extracts a substance having very special and characteristic pharmaco-dynamical properties. In the minds of many writers the adrenal problem has resolved itself into the problem of the chromaphil tissues.

There can be no doubt that adrenine is passed out in certain very small quantities into the general blood-current, and that the output is dependent on the nervous system, and can be modified by the action of certain drugs. Suppression of the adrenin output either by extirpation of one adrenal and denervation of the other, or by the removal of one and a large proportion of the other body and denervation of the remaining fragment, does not affect the life or health of animals (Stewart[116]). There is little or no evidence that the medulla of the adrenal bodies is of fundamental importance as regards the life of the animal. Such experiments, of course, do not show that the tissue has no function.

Cannon believes that the adrenal medulla is stimulated to secrete by emotional excitement, by "pain" and by asphyxia. He finds that after emotional excitement the blood drawn from the inferior vena cava anterior to the opening of the adrenal veins causes inhibition of a beating intestinal strip, whereas that removed before excitement has no such effect. Since excitement after removal of the adrenal bodies does not yield this result, and since the effective blood loses its inhibitory power when exposed to oxygen (a procedure known to destroy adrenine) the inference is drawn that adrenal secretion is stimulated by great emotion. This "emergency theory" is very generally accepted, though strenuously opposed by Stewart and others.

There can be no doubt that the chromaphil tissues can take a part in certain physiological reactions under experimental conditions, as for example when the splanchnic nerve is stimulated. The occurrence of the "dip" in the normal splanchnic curve and its abolition when the adrenal veins are tied, is sufficient evidence of this. On the other hand we

have yet no evidence, direct or indirect, sufficiently convincing to prove that under ordinary conditions the chromaphil tissues are necessary to the efficient working of the body.

7. *The Islets of Langerhans*

It was long ago suggested by Schäfer that the internal secretion of the pancreas is produced by the islets of Langerhans, and in 1916 the same author employed the name "insulin" for the hypothetical substance secreted by the islets. This name has recently been applied by Banting and Macleod to the pancreatic preparation which has the power of reducing the blood-sugar when injected beneath the skin of an animal, and which is now being employed as a remedy for diabetes.

Minkowski and Mehring [98] first showed that complete removal of the pancreas in the dog, cat, and pig is followed by diabetes. That this is caused by the absence of an internal secretion is proved by the facts that it does not occur if the gland be left *in situ* and the duct tied, nor does it occur if a portion of the pancreas be grafted in some situation remote from its normal position (e.g. underneath the skin or in the peritoneum). How the internal secretion of the pancreas normally prevents glycosuria is not yet clear. The evidences that the islets and not the pancreas as a whole are concerned with the internal secretion are as follows: it is stated that in disease of the pancreas associated with diabetes, it is the islets rather than the zymogenous tissue which are affected. When the duct of the gland is tied the zymogenous tissue atrophies and the animal is enabled to survive because the islets do not atrophy. Insulin has recently been extracted from a pancreas after atrophy of tubules, and from the separate islets of certain fishes, where the islets can be obtained free from zymogenous tissue.

It is yet too early to discuss how insulin lowers blood-sugar, or precisely what is the effect on the progress of a case of diabetes. It is only certain that in many cases insulin will restore a diabetic patient to comparative health and comfort, but the treatment has to be continued.

The chemical nature of insulin has not yet been ascer-

tained, nor have its general physiological actions been fully investigated.

It seems clear that the evidences for "internal secretion" on the part of the islets is almost as convincing as in the case of the thyroid.

8. *The Interstitial Cells of the Testis*

When the testes are removed before the age of puberty in man, there is no growth of hair on the face, and the growth of the thorax, pelvis, and larynx is arrested. There is also a tendency to gigantism and the mental characters are affected. There is a hindrance to further growth of the reproductive apparatus. The vesiculæ seminales and the prostate are atrophied.

In various animals if the testes are extirpated early there is no development of secondary sexual characters (see Fig. 35).

Fig. 35.—The Effect of Castration on Horn Growth. *A*. Herdwick Ram (normal). *B*. Herdwick Wether castrated young without horns visible. (Marshall and Hammond.)

If the vasa deferentia are tied it is said that, although the seminferous tubules degenerate, the interstitial cells are unaffected and the animal remains normal. It is only when we remove the atrophied testis with the interstitial cells still in good condition that we get the usual effects of castration. Certain grafting experiments also lend support to the view that the internal secretion of the testis is in fact derived from the interstitial cells.

There has been a vast amount of work in recent years upon the "internal secretions" of the testis, but space will not permit of more than the above sketch. It cannot yet be affirmed with any degree of certainty that the "internal secretion" is a function of the interstitial cells rather than of the testis as a whole. Moreover, we know nothing about the act of secretion, or the chemical nature of the product. Again, we can form no conception of the mechanism by which the circulation of small quantities of a definite chemical substance in the blood can bring out highly specific structural changes in organs and tissues. The problem here is even more complicated than in the case of the thyroid. In the latter instance it is not so remote from our comprehension that a substance should be carried in the blood-stream to different parts of the body and have an influence in accelerating chemical processes (e.g. oxidation). It is true that thyroid substance hastens metamorphosis in amphibians, but it is possible that this is secondary and an inevitable consequence of an increased metabolism.

9. *The Interstitial Cells of the Ovary*

Not much is known of the effects of extirpation of the ovaries before the age of puberty in the human subject. When both organs are removed after the age of puberty, the most marked effect is the cessation of menstruation. There may be also atrophy of uterus, vagina, and external genitals. Nervous and emotional changes are also said to occur. In some female animals removal of the ovary has been said to lead to the appearance of male characters.

Very numerous grafting experiments have been carried out. It is said that if ovaries are grafted into young male guinea-pigs, the mammary glands may develop and secrete milk. This and similar results are more likely to occur if the male be previously castrated.

All these effects are commonly supposed to be due to an internal secretion on the part of the interstitial cells of the ovary, and many other functions are also ascribed to these elements. As in the case of the testis we have no direct knowledge of any secretion or of its chemical or other characters,

nor can we form an idea of how a secretion could modify the growth and structure of organs and tissues.

10. *The Corpus Luteum*

Since removal of the yellow bodies accelerates ovulation and injection of luteal extracts into fowls will prevent that occurrence, the theory that the corpus luteum influences metabolism in such a way as to prevent ovulation during pregnancy seems now to be established.

It is found also that ovariotomy (involving removal of corpora lutea) causes discontinuance of pregnancy. The corpus luteum governs the fixation of the embryo by stimulating the uterine mucous membrane to hypertrophy. To be more accurate, it would appear that the substance derived from the corpus luteum sensitizes the uterine mucous membrane and renders it capable of reacting to mechanical stimulation as, for example, that of the ovum.

There is some evidence to show that the stimulus to the growth of the mammary gland arises in the yellow body.

The same remarks in regard to secretion as were made in regard to the interstitial cells, apply equally to the corpus luteum.

CHAPTER IX

METHODS OF INVESTIGATION IN PROBLEMS OF "INTERNAL SECRETION"

It is to be expected that the processes of "internal secretion" being of a nature so different from those of ordinary secretion would not lend themselves to investigation by methods ordinarily employed in the study of secretion. But a serious attempt to treat "internal secretion" as a process closely akin to ordinary or external secretion has been made by numerous observers. We have already studied the sometimes desperate attempts to make out secretory changes in cells. Investigators have studied stimulation of nerves and electrical changes, as we have already seen. But the conditions and the problems are so different that "internal secretion" has had to employ a variety of methods not employed in the study of glands like the submaxillary or the liver. Most of these methods have already been dealt with incidentally in the preceding chapters, but it may be useful to treat them categorically in this place.

Pathology

There can be no doubt that a large and important part of our information about "internal secretion" has been derived from human pathology and clinical medicine, and these lines of investigation, though among the oldest, are still of very great value.

Extirpation experiments

Extirpation experiments have given valuable information in regard to the thyroids, parathyroids, adrenals, and other glands. But the results of such experiments are often contradictory, for the reason that the technical difficulties are

often very great. It is often impossible to remove the tissue one wishes to remove without doing damage to neighbouring structures. Complete removal of an organ is of course a laboratory experiment and owing to its suddenness is an event which can never happen in pathology. Extirpation carried out in a series of steps at successive operations is more valuable. Better still are operations in which the organs are crushed, damaged, or infected artificially.

Grafting, feeding and subcutaneous and intravenous injections

Grafting. These methods have been extensively employed. It is doubtful whether grafting is ever completely successful in the sense that the grafted tissue lives permanently in its new position. But as a temporary replacement of an essential tissue the proceeding may be very effectual.

Feeding with fresh tissues or extracts has been extensively employed. The therapeutic method called "*organotherapy*" is based upon the principle that the active substance is absorbed unaltered into the circulation. If we seek for cases where it is possible to apply a true substitution-therapy—artificially to replace the "internal secretion" of a gland—we have no clear instances except those of the thyroid and the pancreas. There are, however, some few instances in which extracts of organs, or purified chemical substances obtained from them, are valuable as drugs, apart altogether from the question of "internal secretion." Such are adrenine and extracts from the posterior lobe of the pituitary. Of the rest of the gland and tissue extracts and preparations, it need only be said that in all probability most of them produce no effects of any kind in health or disease. This attitude is based largely on the fact that they produce no effects upon the normal animal, and it is difficult to imagine a drug which is clearly and beyond doubt of value in the treatment of disease and yet which possesses no known pharmaco-dynamical effects. It is conceivable that there may be exceptions to this rule in the case of some of the "internal secretions."

In feeding experiments the effects produced upon metabolism have been carefully studied. The thyroid when given as food has a remarkable influence upon growth and differentiation.

Growth is checked but differentiation (metamorphosis) is stimulated. Adrenal cortex when administered at the same time considerably modifies the effects of the thyroid (Cameron).

Subcutaneous injections of extracts was the method which first aroused modern interest in the subject of internal secretion. The work of Brown-Séquard in 1889 upon testicular extracts was of doubtful value in itself, but it stimulated research and led indirectly to valuable results. Subcutaneous or intra-peritoneal injection of most other glands and tissues has since been carried out. By far the most striking are those obtained by injection of extracts of the chromaphil tissues. In many cases there is not sufficient evidence to warrant us in regarding the effects as evidences of internal secretion.

Intravenous injections. The method of intravenous injection of tissue extracts holds a peculiar position and requires some discussion. This method only came into prominence in 1894, after the publication by Oliver and Schäfer of their discovery of the extraordinary pharmaco-dynamical effects produced by extracts made from the chromaphil tissue contained in the interior of the adrenal body. Since that time numerous observers have investigated the effects of extracts made from every organ and tissue in the body, but the hope of discovering some remarkable activity which might rank in importance with that of adrenin has not been fulfilled. So far as the chief physiological activities of animal extracts are concerned (the effects upon heart, blood-vessels, and certain other tissues) the main points of interest were fully established by Oliver and Schäfer. One other tissue besides the chromaphil—namely, the nervous portion of the pituitary body—contains a *pressor* substance. As for extracts made from other organs and tissues, the generalization slowly became established in the minds of the majority of observers that tissues ordinarily impart to extracts a substance or substances, the most striking action of which, when tested physiologically, is a *lowering* of the blood-pressure. There are, of course, other effects also, as, for example, that on the pupil.

Notwithstanding that investigators were given frequent warning that these results are pharmaco-dynamical only, and bear no necessary relation to any hypothetical internal secretion by the tissues explored, communications continued

to be poured out which either concluded definitely, or implied strongly, that such pharmaco-dynamical results are in themselves evidences of internal secretion. It was clear to the more sophisticated workers that in most, at any rate, of these tissues there is no reason to regard the presence of such active substances as evidence that the tissues in question furnish an internal secretion. It would, for example, be preposterous to assert that the presence of depressor substances in brain extracts has any direct bearing upon the problems of brain physiology ; and no one up to the present time has ventured to suggest that the depressor action of brain extracts points to an internally secreting function of the higher nerve centres. This line of investigation has been pursued with a minimum of critical judgment. Thus Livon divides the glands of the body into two groups, " hypertensive " and " hypotensive," according as their extracts cause a rise or a fall of blood-pressure when injected into the veins of an animal. If we are condemned to make such a classification, it follows that we have to place the chromaphil tissues and the nervous portion of the pituitary body in the first group, and all the other organs and tissues of the body in the second. Gautrelet holds very similar views, and thinks that the " hypotensive " glands owe their activity to the presence of cholin. The point of view of these two observers is typical of those who maintain that all organs and tissues of the body furnish an internal secretion, and that the normal blood-pressure and other standard conditions are maintained by a series of antagonistic chemical agencies arriving from different glands and tissues. This view has been derived from theories of the activity of adrenin in the animal body—theories vitiated by a fundamental error of thought, which was prompted by the belief that the study of the physiological effects of extracts of organs and tissues would solve the problems of internal secretion. It is still necessary to point out that whilst the physiological activity of an extract of an organ or tissue may be the basis of an inference as to internal secretion, the inference cannot become proof until we can show that the active substance is secreted into the blood-stream, and, having arrived there, performs some function in the economy. That it is still necessary to point out these things is shown by a recent

communication of Marfori, in which is contained a careful account of the physiological action of lymphatic glands. This action is the same as that obtained by extracts of brain, spleen, or any other organ or tissue (except chromaphil and posterior pituitary), yet the author, solely on the basis of such action, announces a new hormone, "lymphoganglin."

Gley has attacked the uncritical use of the method of organic extracts, and points out that the attitude we have been discussing is very definite and deliberate in the case of some authors. He quotes Marañon to the effect that one may "consider each extract of an organ as equivalent to the total internal secretion of the gland," and records his astonishment at such views.

To conclude our observations on this part of the subject, it may be admitted that there is no objection to the investigation of the activities of tissue extracts, so long as the investigator realizes that the results are in themselves only of pharmacological interest.* It is only when several other lines of research have been explored that we may draw conclusions about an "internal secretion."

* More striking results might frequently be obtained if investigators used more concentrated extracts.

CHAPTER X

CHEMISTRY OF THE "INTERNAL SECRETIONS"

Thyroid

Two definite compounds containing iodine have been separated from thyroid tissue, a globulin (iodo-thyro-globulin) which exists as such in the gland, and a derivative of tryptophane (*thyroxin*) obtained as a cleavage product and containing 65 per cent. of iodine. The evidence before us points to thyroxin as one of the active compounds of the "secretion" of the thyroid.

Thyroxin. In 1919 Kendall announced the isolation of a definite crystalline iodine compound which he calls "thyroxin." This is said to be 4, 5, 6, tri-hydro, —4, 5, 6, triiodo, —2 oxybetaindolepropionic acid. It exists in three forms :

(1) The keto form with the carbonyl group adjacent to the inimo.
(2) A tautomeric enol form.
(3) A form with an open ring structure (cp. creatine and creatinine).

It may be regarded as a derivative of tryptophane. According to Kendall the third form is that in which iodine occurs in the body.

If nitrous acid be added to an alcoholic solution of thyroxin or to a suspension in water in presence of hydrochloric acid, a yellowish colour is developed which, on addition of ammonia, changes to deep red. This reaction serves as a test for thyroxin. Thyroxin is more susceptible to reduction than to oxidation. Zinc and other metals in acid or alkaline solution split off iodine and break up the nucleus. Mild oxidizing agents have no effect, stronger ones break down the molecule. Thy-

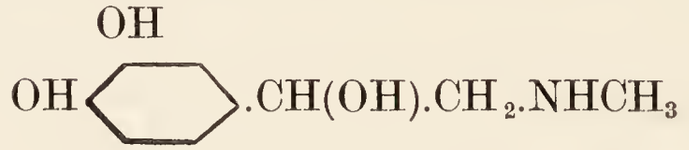
roxin is unstable in sunlight; iodine is split off as hypoiodous acid and subsequently as free iodine.

According to observations of Cameron, Kendall's thyroxin produces effects similar to those of thyroid but quantitatively, compared on a basis of iodine content, the effects of thyroxin are distinctly less than those of dried thyroid. It is clear then that there must be some other active principle. Reid Hunt has recently concluded that the physiological action of thyroxin is only a quarter of that of thyroid.

Thyroxin has not been synthesized.

Chromaphil Tissue

Adrenine, the active substance obtained from the chromaphil tissues, has the following constitutional formula—



It is, therefore, ortho-dioxyphenyl-ethanol-methylamine, and is related to tyrosin-p-oxyphenylamino-proprionic acid.

It can be synthesized, and several synthetic products are now on the market.

It is a colourless crystalline substance, having a melting point of 211–212° C. It is not easily soluble in cold water, but more readily in hot. It is insoluble in most organic solvents, such as alcohol, ether, or chloroform. It is a strong base and is soluble in mineral acids and caustic alkalies, while it is insoluble in carbonates or ammonia. It is not precipitated by alkaloidal reagents such as picric acid, tannic acid, sublimate, phospho-tungstic acid, etc. As a phenol it forms water-soluble compounds with fixed caustic alkalies. It is easily oxidized.

Pancreas (islets of Langerhans)

Insulin. Banting's insulin has not yet been obtained in a state of chemical purity, and not much can be said about its chemical properties.

Insulin is prepared from the pancreas of animals by fractional alcoholic extraction. It dialyses easily. It is almost certainly derived from one or more amino-acids, and when

injected into the blood-stream of normal animals causes rapid fall of blood-sugar. The substance or substances formed have not been ascertained with certainty.

By proper adjustment of dosage of insulin and of the diet, both the hyperglycæmia and the glycosuria and the accompanying ketonuria occurring in diabetes may be kept in abeyance.

Practically nothing is known of the chemical nature of the other "internal secretions."

CHAPTER XI

SECRETION AND "INTERNAL SECRETION"

There has been much loose writing and loose thinking on the whole subject of internal secretion. The lack of precise knowledge has tempted writers to conduct crude inquiries, and to promulgate theories, worthless in themselves, which have occupied much valuable space in the literature.

A great deal would be gained if investigators could be induced to agree that the act of secretion can only be carried out by certain kinds of tissue—namely those which consist of highly specialized epithelial cells. We have seen that many writers, in view of the fact that different organs and tissues have different functions, and consequently, pour out into the blood-stream different chemical substances, have taught that all these tissues are to be regarded as having an internal secretion, and that this secretion is in each case a specific one. But this, as pointed out by Kohn, and as I have already urged on several occasions, is simply a misuse of the term "secretion." I cannot find better words to express my views on this matter than those I have employed in my monograph on internal secretion[121].

"Just as we have certain tissues—namely, muscle and nerve—highly specialized and set apart for the functions of motility, and the conduction of irritability, so we have certain other tissues also highly differentiated and set apart for the purposes of secretion, and it is to these only that we can with propriety ascribe the functions. Such are secretory cells and their accumulations called 'glands.' The secretory cells are in their origin and in their character *epithelial. Secretory cells are highly specialized epithelial cells.* It is not necessary to insist on the criterion in the case of externally secreting glands, because here it is generally recognized, but it is just as important in regard to internal secretion, if the

term is to be defined with anything approaching to accuracy. The morphological sign of special differentiation in gland cells is the presence of granules which undergo periodical changes in number and position according to the stage of activity of the gland. It is not, perhaps, possible to insist on the recognition of granules as definite as those in the pancreatic cells before we admit a structure into the category of internally secreting glands, but it is essential that the constituent cells should have the general character of glandular—i.e. secretory—cells. This is in some instances not altogether an easy matter to determine, and . . . there is still some discussion as to whether such a tissue as the chromaphil may reasonably be supposed to have a secretory function. That a discussion of this kind should arise in connexion with a tissue generally supposed to be internally secretory shows how little we know about the actual act of secretion in such a case.

"We conclude, then, that secretion (internal or external) represents a highly specialized grade of metabolic activity and should be distinguished as rigorously from general metabolism as the contraction of muscle from general motility (Kohn).

". . . We are now in a position to define internal secretion. *The process consists in the preparation and setting free of certain substances of physiological utility (the raw materials for which are supplied by the circulating blood) by certain cells of a glandular type ; the substances set free are not passed out on to a free surface but into the blood-stream.*

"According to this definition, the products of ordinary metabolism, and even special products of metabolism arising in such kinds of highly specialized tissues as muscle and nerve, are excluded from the internal secretions."

It is still desirable to point out that the term "internal secretion" has been used too generally and too confidently. Our knowledge of internal secretion is not yet even remotely comparable in accuracy and definiteness with our knowledge of "external" or ordinary glandular secretion.

It is possible that such an organ as the parathyroid does not pour out or "secrete" anything into the general blood-stream. It may be that toxic substances circulating in the

blood are destroyed or rendered innocuous while the blood containing them is passing through the parathyroid. In this case the toxic materials might pass into the cell and there become destroyed and some newly-formed harmless substance might be returned to the blood. This is presumably what happens to ammonia compounds in the liver.

After a review of the chief facts in regard to secretion and "internal secretion," we cannot avoid the conclusion that the latter process is something of quite a different nature from the former. It may be that it will be possible to retain the term "internal secretion" in some cases, but that in the case of others some new classification of physiological processes will render this term unsuitable. It seems certain that the so-called "ductless glands" do not all function in the same manner, and that only in a few of them can we, even reservedly, speak of a process of "secretion."

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